MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 1997, p. 65–89 Vol. 61, No. 1
0146-0749/97/$04.00+0

Potential Virulence Factors of *Proteus* Bacilli

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INTRODUCTION

Bacterial pathogens have developed many strategies for survival in higher organisms, which during their evolution have formed very sophisticated defense mechanisms. This defense system includes nonspecific reactions such as mechanical clearing of the mucosa, control of iron transfer, phagocytosis, elimination of bacteria by enzyme attack (e.g., by lysozyme), and activation of complement, as well as specific reactions involving antibodies and cells of the immune system. Pathogenic bacteria have worked out many different ways to overcome the host defense system. A number of biological features known as virulence factors are common to many bacterial species, although some of these are characteristic only for certain bacteria (76). Common bacterial properties involved in the infection process include adhesion to epithelial surfaces, invasion (penetration) of host cells, intracellular multiplication of the pathogen, colonization of the cell tissue or transmission to a new susceptible host, production of enzymes which damage the host defense system, and synthesis of toxins (91, 110).

For a long time, scientists have been fascinated by the problem of particular virulence factors characteristic of certain bacteria but have often neglected the complex role of the set of virulence factors developed by them, as well as the complex mechanisms of the host defense. More recently, investigators have paid more attention to detailed examination of bacterial pathogenic factors, which is made possible by the use of different genetic and molecular techniques. These factors can also be studied in animal models. The development of cell line culture techniques enabled us to monitor host-pathogen interactions in vitro. Genetic cloning techniques allowed the identification of clusters of genes which code for virulence factors (37, 41, 205, 273, 282). The results of these studies have made possible a better understanding of the infection process on the molecular level.

Below, we focus on particular pathogenic factors expressed by gram-negative bacteria of the genus *Proteus. Proteus* bacilli have developed several morphological and biochemical features and factors such as fimbriae, flagella, enzymes (urease, proteases, and amino acid deaminases), and toxins (hemolysins and endotoxin), which act individually or in concert during infection (3, 13, 23, 65, 156, 172, 179–181, 309).

GENERAL CHARACTERISTIC OF THE GENUS *PROTEUS* AND ITS ROLE IN INFECTIONS

The first description of *Proteus* rods as putrefying bacteria was made by Hauser in 1885 (93). The author considered their possible role in pathological events in higher organisms. This role was fully confirmed in subsequent decades (311). *Proteus* bacilli are now treated as well-known opportunistic pathogens that cause infections in humans, most frequently in persons with anatomical and physiological defects. It should be stressed, however, that these bacteria are in general less virulent than the pathogenic *Escherichia coli* strains.

The genus *Proteus* belongs to the family *Enterobacteriaceae* (39, 209, 210). The most characteristic feature which distinguishes *Proteus* rods from other members of this family is the swarming phenomenon. Bacteria belonging to *Proteus* spp. (and *Serratia* spp.) exhibit swarming growth (2, 21, 180, 313).

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TABLE 1. Tests useful for differentiating the species and biogroups of *Proteus*[a]

Test or description	*P. vulgaris*		*P. penneri*	*P. mirabilis*	*P. myxofaciens*
	Biogroup 2	Biogroup 3			
Indole production	+	+	−	−	−
Ornithine decarboxylase	−	−	−	+	−
Maltose fermentation	+	+	+	−	+
Salicin fermentation	+	−	−	−	−
D-Xylose fermentation	+	+	+	+	−
Esculin hydrolysis	+	−	−	−	−
Human pathogen	+	+	+	+	−
Insect pathogen	−	−	−	−	+

[a] See reference 100 for details.

According to *Bergey's Manual* (209), *Proteus* consists of four species: *Proteus vulgaris*, *P. mirabilis*, *P. penneri*, and *P. myxofaciens*. The last of these is insignificant in infections of humans and has been isolated from living and dead larvae of the gypsy moth, *Porthetria dispar* (60). *P. penneri* has been proposed as a novel species by Hickman et al. (100) on the basis of DNA-DNA relatedness found among three *P. vulgaris* biogroups. Identification and typing of *Proteus* clinical isolates can be also performed by computerized analysis of electrophoretic patterns of cell proteins (61, 105) or outer membrane proteins and multilocus enzyme profiles (124). Table 1 shows the biochemical classification of these bacteria. Microorganisms belonging to *Proteus* spp. are widely distributed in the natural environment. They can be found in polluted water and in soil and manure, where they play an important role in decomposing organic matter of animal origin. *Proteus* rods also exhibit proteolytic activity under aerobic and facultatively anaerobic conditions. The oxidative deamination of amino acids and the ability to hydrolyze urea to ammonia and carbon dioxide are the most representative biochemical properties of these bacteria. Besides the saprophytic mode of life in the natural environment and in the intestines of humans and wild and domestic animals, *Proteus* bacilli, under favorable conditions, are able to cause pathological events such as nosocomial infections, mainly among geriatric, psychiatric, and paraplegic patients and patients in intensive care units, who are often immunocompromised (55, 69, 129, 247, 274). These kinds of infections are associated with all three opportunistic species, *P. vulgaris*, *P. penneri*, and *P. mirabilis*. However, the last of these is the most common pathogen; this can be explained by its high carriage rate (25%) in human intestines (55). This part of the body is the major reservoir of these bacteria in humans, and this can result in autoinfections or transmission of the bacteria from patient to patient in hospitals (55).

Proteus bacilli play a particularly important role in urinary tract infections (UTI), which can be subdivided into two categories: hematogenous infections (also known as systemic infections) and ascending infections, in which bacteria colonize, step by step, the introitus, urethra, bladder, ureter, and, in the end, the kidneys (241). The second type of UTI is more common to *Proteus* strains. *P. mirabilis* most frequently causes UTI in patients with urinary catheters in place (303, 304) or with structural abnormalities (281), as well as after surgical intervention in the urinary tract. Warren (302) showed that *P. mirabilis* is the third most common (after *Escherichia coli* and *Klebsiella pneumoniae*) cause of complicated UTI (causing 12% of infections) and the second most common (after *Providencia stuartii*) cause of catheter-associated bacteriuria in the group of long-term catheterized patients (causing 15% of infections). UTI are also caused by the two other species, *P. vulgaris* and *P. penneri* (210, 253). *Proteus* UTI are known to be frequently persistent, difficult to treat, and even fatal, depending on the severity of illness in particular patients. The complications of infection in catheterized and noncatheterized patients include the development of urolithiasis, urinary tract obstruction, obstruction of urinary catheters, bladder and kidney stone formation, and bacteriuria (145, 247, 258, 274, 304). *Proteus* bacilli, when present in the kidneys, can cause severe histological damage, characterized as acute pyelonephritis (72). It was found by Larsson et al. that *Proteus* rods cause UTI among young boys whereas urinary tract infections caused by *E. coli* are more common in girls (150, 152).

Besides UTI, *P. mirabilis* and *P. vulgaris* have been described as opportunistic etiological agents in infections of the respiratory tract and of wounds, burns, skin, eyes, ears, nose, and throat, as well as in gastroenteritis resulting from the consumption of contaminated meat or other food (53, 59, 210). The possible role of *Proteus* in diarrheal diseases was studied in the 1950s and was later investigated in studies of children (82, 149, 278, 300), adults (96, 246), and birds (17). Epidemiological and serological investigation confirmed the presence of *Proteus* and/or *Providencia-Morganella* strains in the stools of the patients. However, the problem remained unsolved, for two reasons. There was no proof that any of the isolated *Proteus* serotypes could initiate epidemic disease in infants, like *E. coli* pathogenic strains did, and other possible etiological agents in the feces were not always studied and ruled out. It is also postulated that these bacteria may play a role in rheumatoid arthritis (RA) since specific anti-*Proteus* antibodies were found in patients with active RA (64, 70, 314). Recently, Senior et al. (256) analyzed by enzyme-linked immunosorbent assay 100 rheumathoid factor (RF)-positive serum samples and 100 RF-negative serum samples from patients with various autoimmune diseases. They found that sera from patients with clinically proven RA contain elevated levels of immunoglobulin M (IgM) but not IgG antibodies to *P. mirabilis* with respect to RF-negative sera. Sera from the RA patients contain greater amounts of IgM antibodies to *P. mirabilis* than to the other microorganisms. In these sera, significantly higher levels of IgA antibodies to *P. mirabilis* were also found. The authors show that the IgM response in RA patients was associated with all 11 different O serotypes of *P. mirabilis* tested and with those of other *Proteus* spp.

Since *P. penneri* has been created as a new species in the genus *Proteus*, a short description is included. *P. penneri* has the general characteristics of the genus *Proteus* such as production of hemagglutinins and fimbriae (306) and synthesis of hemolysins (159, 161, 236, 239), IgA proteases (156, 250), and urease (187). The biochemical properties of the last enzyme are unique, and it could be distinguished from the ureases of

TABLE 2. Virulence factors of *Proteus* bacilli

Virulence factor	Contribution to the pathogenicity	References
Fimbriae	Adherence of bacteria to the epithelial tissue	13, 14, 34, 168, 242, 315
Flagella (swarming phenomenon)	Ascension of bacteria from ureter to the kidneys	3, 4, 21, 22, 180
Urease	Elevation of pH in the surrounding of bacterial growth resulting in stone formation; cytotoxicity against HRPTEC	109, 111, 162, 185, 187, 188
IgA proteases	Disruption of IgA	155, 156, 250, 254, 255
Amino acid deaminases	Production of α-keto acids acting as siderophores	65
Invasiveness	Penetration and internalization of bacteria into host cells	54, 207, 238
Hemolysins (HpmA and HlyA)	Cytotoxicity	37, 181, 187, 239, 309
CPS	Formation of biofilm and stones	68, 218
LPS (endotoxin)	Endotoxicity; resistance to the human serum	151, 152, 153, 195, 229, 231

Providencia, *Morganella*, and other *Proteus* spp. The etiological role of *P. penneri* in UTI was confirmed (72, 144, 145); moreover, the presence of this species in fecal specimens from healthy people and patients with gastrointestinal syndromes and from birds was also shown (17, 193, 246). Engler et al. (72) isolated *P. penneri* from patients with acute lymphocytic leukemia. The resistance of *P. penneri* strains to chloramphenicol distinguished them from the other *Proteus* bacilli (74, 77, 100). The susceptibility of *P. penneri* strains to antimicrobial agents has been investigated by many laboratories (77, 83, 95, 97, 216). It seems that resistance of these bacteria to antibiotics is determined chromosomally rather than by plasmids (160).

POTENTIAL VIRULENCE FACTORS OF *PROTEUS* BACILLI

Potential virulence factors, as well as features of *Proteus* rods important in infection processes, are specified in Table 2. Brief descriptions are presented below.

Surface Structures

In interactions between gram-negative bacterial pathogens and higher organisms, a significant role is played by surface structures of bacteria (e.g., fimbriae, flagella, capsule antigens, and outer membrane components), as well as by host tissue factors. It is believed that the cell surface of *Proteus* bacilli is very important to their virulence, particularly their adherence ability, colonization of the urinary tract, and formation of stones.

Fimbriae and adherence ability. Bacterial adhesion to epithelial surfaces is thought to be one of the most important virulence factors (227, 245), playing a significant role in the initiation of UTI. Bacterial uropathogens (*E. coli*, *Proteus* spp., and *Streptococcus* spp.) exist mostly in the intestinal tract, from where they colonize the periurethral region and then ascend into the bladder, causing symptomatic or asymptomatic bacteriuria. The data obtained by several authors suggest that adhesion of bacterial cells to uroepithelial cells is very important in this process in infections caused by *Proteus* spp. (47, 243, 283).

The bacterial adhesion capacity is most frequently associated with the presence of fimbriae on bacterial cells. It has been shown that fimbriae are indeed responsible for the attachment of *Proteus* bacilli to uroepithelial cells. Silverblatt (270) observed in an experimental ascending infection that a heavily fimbriated *P. mirabilis* strain caused pyelonephritis with higher efficacy than a lightly fimbriated one did. By electron microscopy, it was possible to see bacteria with fimbriae bound to renal pelvic mucosa. In contrast to this effect, fimbriae diminished the ability of *P. mirabilis* rods to infect the renal parenchyma by the hematogenous route (272). Studies in vitro have shown that fimbriae enhance the binding of bacterial cells to uroepithelial cells but render the pathogen more susceptible to phagocytosis. Quantitative assay showed that heavily fimbriated strains were more strongly bound to the exfoliated rabbit bladder cells than lightly fimbriated bacilli were. The opposite results were obtained when the phagocytosis of both kinds of strains was investigated. The bacteria with large numbers of fimbriae were readily ingested by polymorphonuclear cell monolayers, whereas the bacilli with small numbers of fimbriae were resistant to phagocytosis. It was also shown that a *Proteus* strain that was originally strongly fimbriated became resistant to phagocytosis after it had been defimbriated (272).

Previous ultrastructural studies (40, 66, 272) of *Proteus* strains have shown two types of fimbriae—thick (approximately 7 nm in filament diameter) and thin (4 nm in diameter). The first type, also known as type IV fimbriae, was found to be mannose resistant and was named *Proteus*-like fimbriae (MR/P). The second type is the type III fimbriae, which are mannose-resistant *Klebsiella*-like fimbriae (MR/K). These types of fimbriae are associated with their ability to hemagglutinate untreated (MR/P) or tannic acid-treated (MR/K) erythrocytes from several animal species (1, 57, 78, 202, 203). The MR/K phenomenon was associated only with the hemagglutination pattern of *Proteus* and was loosely correlated with the expression of fimbriae shown by electron microscopy analysis. Since no direct correlation with any of genetically characterized *Proteus* fimbriae was found, we will use the term "MR/K hemagglutinins" rather than "MR/K fimbriae" in this review. The presence of mannose-binding type I fimbriae on *Proteus* cells is not settled. Some authors have found them on these bacteria but only rarely expressed by strains from UTI (2, 11, 67, 202, 259). Hornick et al. (106) showed that these types of fimbriae are absent in *P. mirabilis* isolates. It has also been found that in *Morganella*, *Proteus*, and *Providencia* spp., the different types of fimbriae may exist simultaneously. Thus, it is very difficult to identify and characterize them separately. Electron microscopic examination of bacilli from cultures giving hemagglutination showed at least six distinct fimbrial types (202).

Silverblatt and Ofek (272) demonstrated that MR/P fimbriae are more important for the adherence of bacteria to epithelial cells than MR/K hemagglutinins. Strains possessing MR/K hemagglutinins adhere no better to these cells than do depiliated bacteria. This adhesion capacity does not depend on the type of host cell; the fimbriae were bound to human buccal epithelial cells, as well as to rabbit bladder cells. Moreover, it was found that in rats infected transurethrally with *P. mirabilis* rods expressing either MR/P or MR/K hemagglutinins, a higher frequency of cortical abscess was observed within 1 week in the rats infected with strains having MR/P fimbriae than in those infected with MR/K strains (271). Taken to-

TABLE 3. Fimbriae of *Proteus* rods and their importance in pathogenicity

Type of fimbriae	Contribution to the pathogenicity	Reference(s)
MR/P	Colonization of the upper part of the urinary tract	14
MR/K	Association with the adhesion of strains to catheters	183, 316
PMF	Colonization of bladder but not kidneys	168
ATF	Not studied	
NAF	Not studied	
P. mirabilis P-like fimbriae	Not studied	

gether, the results of these studies suggest that MR/P fimbriae may play a more important role in the development of pyelonephritis than do MR/K hemagglutinins. This suggestion was confirmed by others, who found that mice immunized with purified fimbriae of uropathogenic *P. mirabilis* strains were protected against infection caused by homo- and heterologous strains (154).

Intensive studies carried out mainly by Mobley's group have enabled us to draw our conclusions about *Proteus* fimbriae and nonfimbrial adhesins (Table 3).

(i) MR/P fimbriae. The MR/P fimbriae were isolated and purified for the first time by Sareneva et al. (242) from *P. mirabilis* 3087 and appeared to be 21-kDa proteins. This fimbrial material had the best affinity to the urethral epithelium of the lower part of the urinary tract in vitro. The adhesion phenomenon could be completely inhibited by the Fab fragments of antibodies against the purified MR/P fimbriae (242).

MR/P fimbriae were also characterized by Bahrani et al. (13, 15). The major subunit of these fimbriae, designated the MrpA protein, has a molecular mass of 18.5 kDa and is encoded by a 525-bp open reading frame (*mrpA*). MrpA was predicted to be a 175-amino-acid polypeptide with a 23-amino-acid hydrophobic leader sequence. It was found that MrpA was similar to uroepithelial cell adhesin (315) (these two proteins possess identical fragments of 10 amino acids) but not to the MR/P fimbriae isolated by Sareneva et al. (242). The *mrpA* gene was transferred to *E. coli* HB101, after which these bacteria expressed on their surface MR/P fimbriae that were recognized by MR/P-specific monoclonal antibodies and agglutinated erythrocytes in a mannose-resistant manner. The isolated MrpA polypeptide showed a strong similarity to SmfA protein, the structural subunit of mannose-resistant fimbriae of *Serratia marcescens* (57% amino acid sequence identity, particularly in the N and C termini) (16). It is speculated that gene organization of MR/P fimbrial sequence may be similar in some respect to that of P fimbria operon in *E. coli* (13). The other genes also involved in the determination of MR/P fimbriae have been identified. The *mrp* gene cluster is organized similarly to that of other fimbrial operons and predicts eight polypeptides (Fig. 1). The *mrp1* gene is upstream of the *mrpA* gene and is transcribed in the direction opposite to the rest of the operon. The *mrpB* lies downstream of *mrpA* and is followed by *mrpC*, which determines the outer membrane "platform" polypeptide. Downstream of *mrpC* is *mrpD*, predicted to determine a chaperonin protein whose role is to carry the major and minor fimbrial subunits to MrpC. *mrpD* is followed by three genes, *mrpE*, *mrpF*, and *mrpG*, encoding minor fimbrial subunits (16). By use of the Swiss Prot and PIR databases, Bahrani and Mobley (16) have shown that all eight predicted polypeptides share ≥25% amino acid identity with at least one of the other fimbrial proteins determined by the *fim*, *pap*, *smf*, *fan*, and *mrk* operons. No homology between MR/P fimbrial proteins and any nonfimbrial polypeptides has been found.

MR/P fimbriae are strongly immunogenic. Mice infected transurethrally with MR/P *P. mirabilis* strains produced specific antibodies whose level increased in the chronically infected animals (13). To investigate the contribution of MR/P fimbriae to colonization of the urinary tract, the MR/P fimbrial mutant was constructed (14). The facts show that the *mrpA* mutant failed to express MR/P fimbriae and that it was not able to agglutinate erythrocytes, confirming the previous opinion that MR/P fimbriae are MR/P hemagglutinins. Using the CBA mouse model of ascending UTI, it was shown that MR/P fimbriae contributed to the renal infections by facilitating colonization of the upper part of the urinary tract. The *mrpA* mutant was recovered in significantly smaller numbers from the urine, bladder, and kidneys. It caused less severe damage to the uroepithelium than did the parent strain, and it did not cause pyelonephritis. The results of this study also showed that additional virulence factors contributed to the pathogenesis of the upper part of the urinary tract, since the loss of MR/P fimbriae by bacterial cells did not completely abolish the colonization of kidneys (14).

(ii) MR/K hemagglutinins. The MR/K hemagglutinins are completely different from the MR/P fimbriae in the tissue-binding pattern. MR/K hemadhesins bound strongly to Bowman's capsule of the glomeruli and to the tubular basement membranes and did not adhere to epithelial cells of urinary

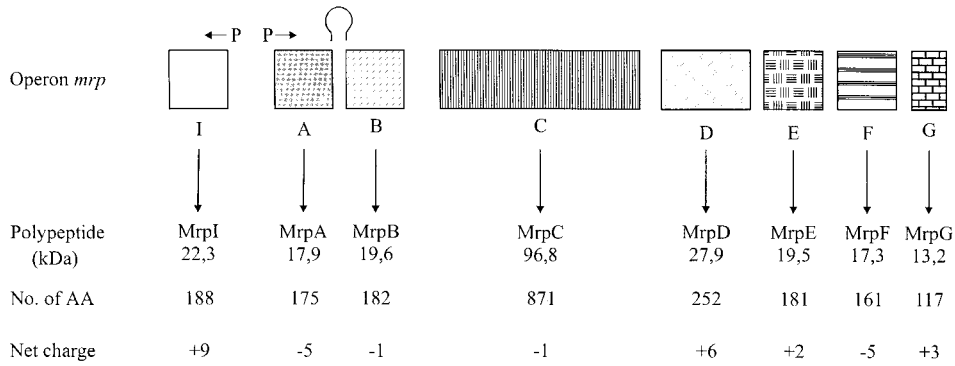


FIG. 1. MR/P fimbriae showing the genetic organization of the *mrp* operon and characteristics of the predicted MR/P fimbrial polypeptides. AA, amino acids. P indicates putative consensus sequence promoters. Arrows show the predicted directions of transcription. A stem-loop structure is predicted to follow *mrpA*. Values shown are for unprocessed polypeptide. Reprinted from reference 16 with permission.

sediment. They do not appear to be homogeneous, and it is not known if the peptides observed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis correspond to a different variant of MR/K hemagglutinins or to different agglutinin types or both (242). MR/K hemadhesins are more characteristic of *P. penneri* than of *P. mirabilis* strains (316). It was shown that 12 of 18 *P. penneri* strains studied formed only MR/K hemagglutinins, which were associated with type III MR/K fimbriae, while 2 strains synthesized both MS (mannose-sensitive) fimbriae and MR/K hemagglutinins and 4 strains produced both MR/K hemadhesins and MR/P fimbriae simultaneously. No *P. penneri* strains which produced MS or MR/P fimbriae alone were found. MR/K hemagglutinins from *P. penneri* E180 were isolated and purified. They represented at least two antigenic types; however, they were antigenically distinct from those of fimbriae from *Klebsiella* spp. (203).

MR/K agglutinins are strongly associated with the adhesion of strains to catheters and with persistence of catheter-associated bacteriuria. It was detected previously in *Providencia stuartii* infections in catheterized elderly patients (183). Roberts et al. (232) observed that of all gram-negative bacteria, *P. mirabilis* bacilli showed the greatest adherence to catheters; the most marked attachment was noticed to red rubber catheters. A high affinity of *P. mirabilis* strains to the polymer substrata used in catheter production e.g., ethylene, propylene, polystyrene, and sulfonated polystyrene, was observed (98). Significant adherence of *P. mirabilis* strains was also found to polymethyl methacrylate (an artificial material used in arthroplasty) and gentamicin-containing polymethyl methacrylate, which were covered by biofilm produced by *Staphylococcus epidermidis* (48). In contrast, *P. mirabilis* bacilli do not adhere to the Tamm-Horsfall protein, which contains mannose glycoproteins, binding the type I fimbriae. Tamm-Horsfall protein is produced in the kidneys and then released into urine together with the bacteria attached by MS fimbriae. The prevalence in the formation of MR/K hemagglutinins in the absence of MS fimbriae, as is strongly manifested in *P. penneri* strains, is probably common to all *Proteus* bacilli and is responsible for the persistence of these bacteria in long-term chronic infections of the urinary tract (316). Further studies involving more sensitive techniques could clear up this problem.

(iii) PMF fimbriae. Previous observations suggested that the MR/K fimbrial subunit was a 19.5-kDa protein, since it was found in material isolated from the surface of bacteria able to agglutinate tannic acid-treated erythrocytes (12). However, the lack of homology between the *pmfA* gene, encoding this protein, and the *Klebsiella mrkA* gene, as well as the lack of significant similarity of the predicted amino acid sequence of MrkA protein (*K. pneumoniae* major fimbrial subunit, 21.5 kDa) to the PmfA protein, indicated that this last one represented the other type of fimbriae, which was termed PMF (*P. mirabilis* fimbriae).

The gene encoding PMF was isolated and sequenced; a 552-bp open reading frame predicts a 184-amino-acid polypeptide, including a 22-amino-acid hydrophobic leader sequence. The unprocessed and processed polypeptides are predicted to be 18.9 and 16.7 kDa, respectively. The predicted amino acid sequence of PmfA protein demonstrated significant amino acid similarity to the *S. marcescens* SmfA fimbrial subunit (35% exact matches) and displayed only 15% exact matches with the predicted amino acid sequences of *K. pneumoniae* MrkA fimbrial protein (12). The genetic organization of the *pmf* region has been shown. The *pmf* gene cluster predicts five polypeptides: PmfA (18.9 kDa), PmfC (93.1 kDa), PmfD (28.2 kDa), PmfE (38.9 kDa), and PmfF (19.7 kDa) (179).

To study the possible role of PMF in the virulence of *P. mirabilis*, a *pmfA* mutant which does not express the 18.9-kDa protein was constructed and used in the CBA mouse model of ascending UTI (168). This mutant colonized bladders of mice at an 83-fold-lower rate than that of the wild-type strain. This shows that PMF may recognize and adhere to a specific receptor on the bladder. Since the *pmfA* mutant colonizes the kidneys in numbers similar to those of the wild-type strain, other fimbriae or hemagglutinins may be important for adherence to kidney epithelium. It was also found that this mutant could adhere to uroepithelial cells as well as the parent strain could. All these data suggest the important role of PMF in colonization of the bladder but not of the kidneys.

(iv) Ambient-temperature fimbriae. Ambient-temperature fimbriae (ATF) were purified and characterized by Massad et al. (167). These fimbriae possess as a major subunit a 24-kDa polypeptide whose N-terminal amino acid sequence of the processed form does not demonstrate similarity to that of any other fimbriae or other protein in the Swiss Prot database. By use of electron microscopy and immunogold labelling of bacterial cells, it was shown that ATF were recognized, by a specific antiserum, as rod-like organelles on the surface of bacteria. The expression of ATF was not correlated with hemagglutination in the manner of MR/P or MR/K. ATF were found on all eight strains tested, and their expression was affected by bacterial growth conditions. The optimal expression of this type of fimbriae was observed in static growth cultures in Luria broth for 48 h at 23°C. The synthesis of ATF was also seen at 37°C in shaking or static Luria broth cultures, at 42°C in liquid minimal medium shaken at 37°C, or on solid medium. The ATF genes were isolated, and a partial DNA sequence was determined. The polypeptides predicted are AtfA (19.0 kDa, a fimbrial subunit) and AtfB (a chaperonin-like polypeptide) (179). ATF may play a role in the growth of *Proteus* bacilli in the natural environment.

(v) Uroepithelial cell adhesin. Wray et al. (315) isolated and identified a protein, termed uroepithelial cell adhesin (UCA), from a uropathogenic isolate of *P. mirabilis* HU 1069. This adhesin was found to be responsible for the attachment of bacteria to uroepithelial cells. The morphological organization of UCA resembles a rod-shaped structure, typical of fimbriae that are also visible on intact organisms. Recently, Bijlsma et al. (34) have shown by electron microscopy that UCA synthesized by UTI-associated *P. mirabilis* strains isolated from dogs corresponds to thin fimbriae with a diameter of 4 nm. The greatest expression of this adhesin took place during the maximal adherence capability of bacteria to epithelial cells, which also suggests that the investigated protein acts as fimbriae, responsible for bacterial attachment to the uroepithelium. Its amino acid composition proved to be similar to that described for *E. coli* fimbriae, and the major fragment of this protein responsible for adhesion had a molecular mass of 17.5 kDa. The N-terminal amino acid sequence of UCA revealed limited homology to K99 fimbriae of *E. coli*. The reason for this is not clear, since these two adhesins attach to different types of tissue: K99 attaches to the intestinal epithelium of calves and sheep erythrocytes, and UCA attaches to desquamated epithelium from the human urinary tract. Therefore, less homology was observed between UCA and P fimbriae, as well as between UCA and type I fimbriae of *E. coli*. These findings may suggest that UCA was formerly an adhesin for the intestinal epithelium (315).

Recently, *P. mirabilis* UCA genes (*ucaA*) have been cloned into *E. coli* K-12 and the DNA sequence of the *ucaA* gene has been determined (58). The UcaA protein deduced from the DNA sequence displays attributes common to many pilins: a signal sequence at the N terminus, a cysteine loop in the amino

half of the polypeptide, and a penultimate tyrosine at the C terminus. The nucleotide and deduced amino acid sequences of UcaA are similar to those of *E. coli* major-subunit fimbriae F17A and F111 (56 and 58% identity, respectively), which suggests that UcaA and these fimbriae have a common ancestor. The amino acid sequence homology has also been found between the UcaA protein and the amino end of the G hemagglutinin of pyelonephritogenic *E. coli*. Significant amino acid similarities have also been demonstrated between the UcaA protein and pilin proteins of *Haemophilus influenzae*, *Bordetella pertussis*, *Klebsiella* spp., and *E. coli* type Ic (34, 33, 31 and 28% identity respectively). However, this has not been reflected in DNA sequence homology. These results suggest functional similarity but less genetic relatedness than between UcaA and the fimbriae mentioned above.

In the genetic studies performed by Bijlsma et al. (34), it was demonstrated that the complete *ucaA* gene of 540 bp encodes a polypeptide of 180 amino acids, including a 22-amino-acid signal sequence peptide, and the molecular mass of the processed polypeptide containing 158 amino acids was calculated to be 16 kDa. The *ucaA* gene was found in all 26 *P. mirabilis* strains tested. None of the isolates other than *P. mirabilis* was able to react with the *ucaA* probe.

Recently, the name "nonagglutinating fimbriae" (NAF) has been proposed for UCA to distinguish it from the other *P. mirabilis* fimbriae (287). It was also shown that the N-terminal sequence of *P. mirabilis* NAF is distinct from MR/P fimbriae, as well as from ATF and PMF. Moreover, it was demonstrated in vitro that the adherence of *P. mirabilis* bacilli to the HEp-2 cell line could be inhibited by preincubation of bacteria with monoclonal antibodies against NAF. This suggests an important role of that type of fimbriae in infections caused by these microorganisms.

(vi) *P. mirabilis* P-like fimbriae. The presence of P-like fimbriae on *P. mirabilis* canine isolates was reported by Bijlsma et al. (34), who identified the *pmpA* gene as a 549-bp fragment of DNA encoding a polypeptide containing 183 amino acids, including a 23-amino-acid signal sequence. This gene was found in 24 of 26 *P. mirabilis* strains but in none of the *P. vulgaris*, *Morganella morganii*, and *Providencia rettgeri* strains used in these studies. The PmpA protein showed a close similarity to the pyelonephritis-associated fimbria (Pap)-like major protein subunit of uropathogenic *E. coli* strains.

The contributions of particular fimbriae or hemagglutinins to the pathogenicity of *Proteus* bacilli are listed in Table 3.

Flagella and swarming motility. In general, the presence of flagella on the surface of pathogenic and opportunistic bacteria has been thought to facilitate the colonization and dissemination from the initial site. The association of motility with the virulence of flagellated gram-negative bacilli like *Vibrio cholerae* (87) and *Pseudomonas aeruginosa* (174, 191) has already been demonstrated.

Proteus bacilli are dimorphic bacteria. When grown in a liquid medium, these cells display swimming behavior and have a distinct morphology; i.e., they are motile, peritrichously flagellated (6 to 10 flagella per cell) rods, 1.0 to 2.0 μm in length. These bacilli, referred to as swimmer cells, are similar in many aspects of their physiology to other members of the family *Enterobacteriaceae*. When transferred to a solid medium, *Proteus* bacilli undergo morphogenesis to swarmer cells and swarm over the surface of solid medium. This kind of growth of *Proteus* rods on solidified nutrient medium is termed the swarming phenomenon (10, 21, 139, 180, 313). Swarming as a form of bacterial translocation across the solid surface of artificial or natural media is characteristic not only for *Proteus* spp. but also for the gram-negative bacteria *Vibrio* spp. and *Serratia* spp. as well as the gram-positive microorganisms *Bacillus* spp. and *Clostridium* spp. (2, 99, 170).

The swarming growth can be simply described as a differentiation of short rods into nonseptate, multinucleate swarmers 20 to 80 μm in length, which is accompanied by 50- to 500-fold increase in the number of flagella, depending on the size of the individual swarmer cell (Fig. 2). The newly synthesized flagella in swarmers are composed of the same protein (36.7-kDa flagellin) as are the flagella in the swimmer cells (25). The morphological conversion of swimmers to swarmers coincides with some marked structural and biochemical changes. For example, the number of nucleoids in swarmers is proportional to the increase in length, and it was found that a 40-μm swarmer cell has about 20 chromosomes (21). In swarmer cells, lipopolysaccharides (LPS) with long O-antigenic side chains predominate, whereas short bacilli synthesized both kinds of LPS with a long and a short O chain. However, the major fraction of this LPS is composed of low-molecular-weight material (269). The outer membrane (OM) of swarmers exhibits higher fluidity than does the OM of the swimmers. There are also differences in the levels of some proteins and expression of some enzymes, e.g., tryptophanase, phenylalanine deaminase, and urease, as well as HpmA hemolysin (6, 24).

The swarming phenomenon is periodic in nature and consists of three phases. Besides the differentiation mentioned above, migration of bacterial mass and consolidation also occur (21). Quite recently, it has been proven that *Proteus* swarming represents a strictly multicellular character. An individual cell, separated from the bacterial population, is unable to move on the agar plate unless it is engulfed by other swarmers. Thus, swarming can be considered a surface-induced, coordinated multicellular differentiation process. This phenomenon begins when a group of swarmer cells migrate rapidly and coordinately away from the colony on solid medium, and it continues until the number of swarmers is reduced either by a loss of cells on the way or when the mass of bacteria changes the direction of motion (21). The cessation of movement is accompanied by division into the short, vegetative swimmer cells. This phase is termed consolidation. The process of differentiation of swimmers to swarmers, migration of the swarmer population, and consolidation is then repeated several times until the surface of the agar medium is covered by concentric rings formed by swarmers during their movement from the central colony to the periphery of the plate. Swarmer cells are formed only when bacteria grow on solid medium; when removed from this and transferred to liquid medium, swarmers dedifferentiate to swimmers by formation of septa, cell division, and reduction of synthesis of flagella (21).

An extracellular slime material was postulated by Stahl et al. (277) as a factor which facilitates swarmer cell translocation, since it was made preferentially by these type of cells. Recently, Gygi et al. (90) showed that translocation of a swarmer cell population of *P. mirabilis* on a solid medium is facilitated by cell surface polysaccharide, enriched in galacturonic acid and *N*-acetylgalactosamine, probably by the reduction of surface friction. This capsular polysaccharide (CPS) seems to be different from the O-specific part of LPS, since the latter does not contain the sugars mentioned above. Genetic analysis of mutants unable to produce CPS on the bacterial surface and of the wild-type strain showed that the mutation is located within a 1,112-bp gene encoding enzymes important for LPS synthesis and assembly of a surface polysaccharide. The gene *orf2*, which encodes a predicted 41-kDa protein, has been named *cmfA* (for capsular migration factor). The Cmf CPS is probably a type of II CPS linked to the phospholipid anchor present in the OM of bacteria.

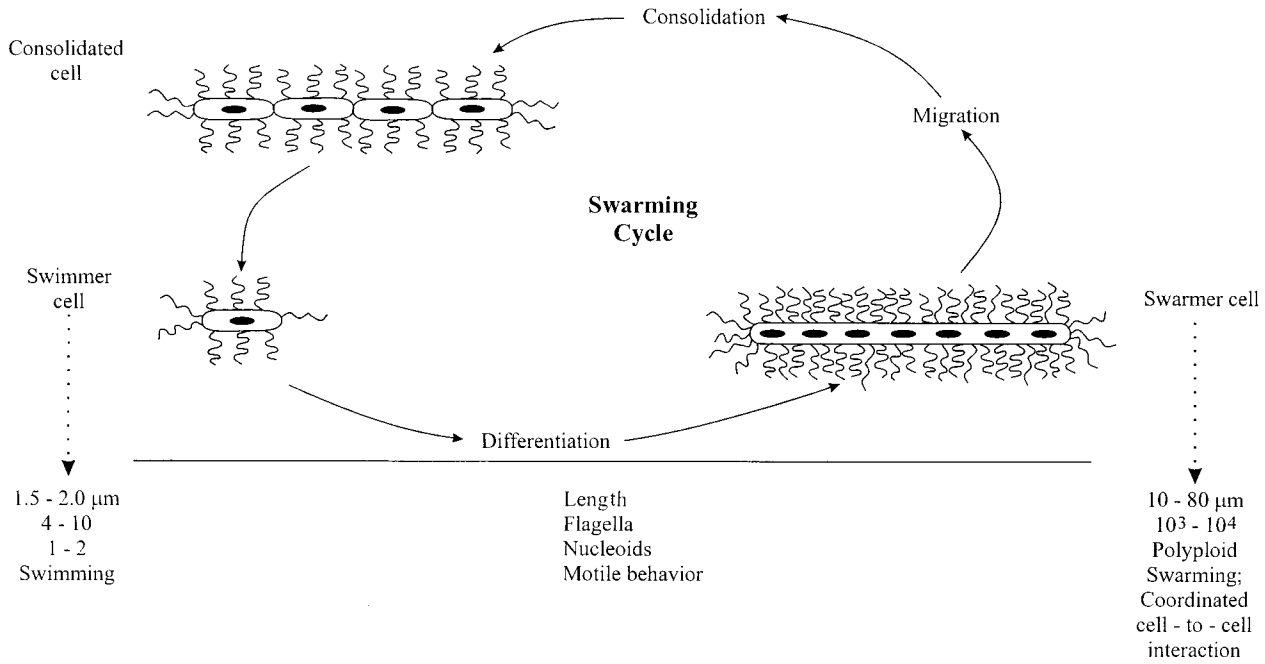


FIG. 2. Swarming cycle of *Proteus* bacilli growing on solid media (6, 21, 23, 180). Swarmer cell differentiation and swarming motility are cyclic. A short, vegetative bacillus (swimmer cell), as observed in liquid culture, differentiates on solid media into the highly flagellated, polyploid, elongated swarmer cell. This process is controlled through the environmental signals, e.g., reduction of flagellum rotation and/or glutamine, a specific chemical stimulus. Then, the population of swarmer cells migrates rapidly and coordinately away from the initial point of inoculation until the number of swarmer cells is reduced. At the consolidation stage, swarming motility is stopped and swarmer cells, after dedifferentiation, revert to a swimmer cells, i.e., short rods.

The genetic basis of the *P. mirabilis* swarming phenomenon was studied by transposon mutagenesis. The transposon Tn*5* was used by Belas et al. (25) to obtain mutants defective in swarming motility. By using this approach, two classes of mutants were revealed. The first class (Srw^{-} mutants) was completely unable to swarm because of defects in the synthesis of flagella, in their rotation, or in surface-induced elongation. The second class consisted of flagellated mutants defective in control and coordination of multicellular motility and was termed Swr^{cr} (swarm-crippled mutants); these mutants had an aberration in their swarming motility. Analysis of mutants defective in swarm cell differentiation and swarming motility provided results which suggested that approximately 40 to 60 genes were involved in *P. mirabilis* multicellular swarming behavior. The marked differentiation of mutants of a broad group, Swr^{cr}, into phenotypic classes can be a base for speculation on the particular mechanisms which resulted in the observed differences (21). Recently, Belas et al. (27) published the results of an analysis of 12 Tn*5* transposon mutants defective in swarmer elongation. They found that a disorder in the function of genes determined flagellar synthesis, LPS and peptidoglycan production, and cell division, as well as synthesis of proline peptidase reflected during swarmer cell elongation.

Analysis of transposon Tn*5* *phoA* mutants of a uropathogenic strain, *P. mirabilis* U 6450, showed that at least 45 genes were involved in controlling motility, cell differentiation, and multicellular behavior of swarmer cells (21, 24, 25). The mutants obtained in this way were flagellated and motile but unable to elongate, or, when forming swarmer cells, they displayed aberrant multicellular migration or consolidation. In the detailed genetic study, eight different mutated genetic loci were identified within the phenotypic classes of mutants. It was demonstrated that a distinct phenotype resulted from mutations in different genetic loci.

Allison et al. (6) have demonstrated that in contrast to the vegetative swimmers, *P. mirabilis* swarmer cells show a substantial increase in the activity of intracellular urease, extracellular hemolysin (HpmA [hemolysin *Proteus mirabilis*]), and metalloproteases. Expression of the genes encoding the synthesis of these pathogenic factors is coordinated in swarmer cells. The ability to invade human uroepithelial cells by *P. mirabilis* swarmers, described for the first time (3), is discussed below. Quite recently, Allison et al. (4) showed that swarming differentiation occurs in vivo and that the differentiated cells are the virulent forms of *P. mirabilis*.

A very important development in the understanding of the swarming phenomenon was the discovery of a signal molecule which initiates cell differentiation and migration. Allison et al. (5) convincingly demonstrated that of the 20 common amino acids, only 1, glutamine, was able to initiate the process on a minimal growth medium. After supplementation with glutamine, which did not support swarming migration, the typical filamentous forms synthesizing high levels of flagellin and hemolysin were isolated from the edges of colonies. The glutamine analog gamma-glutamyl hydroxamate inhibited swarming but not growth of *P. mirabilis* on glutamine-containing minimal growth medium. Transposon mutants defective in glutamine uptake exhibited a response to signal molecule and inhibition by gamma-glutamyl hydroxamate. This can suggest that differentiation created by glutamine signaling can be transduced independently of the well-known glutamine transport system. As compared with swimmer cells, levels of mRNA transcribed from the hemolysin (*hpmA*) and flagellin (*fliC*) genes were markedly increased in swarmers during glutamine-dependent differentiation. The authors consider glutamine to be a specific chemoattractant for swarming cells.

The high viscosity of the bacterial environment and the possible presence of antiflagellar antibodies in the growth medium

are other types of signals involved in swarmer cell differentiation. Both inhibit normal rotation of the flagellar filament, resulting in tethering of flagella and abnormal expression of swarmer cell differentiation (23, 89). From this observation, it can be assumed that the flagella function as tactile sensors of the external condition of growth which directly transfer the signals from the outside into the cells. Gygi et al. (89) analyzed *P. mirabilis* mutants defective in *flhA* synthesis and showed that the mutation affects the function of FlhA (a protein belonging to a family of proteins that are required for flagellar biosynthesis and are implicated in different cellular processes, including the synthesis of virulence factors), leading not only to the lack of motility of bacteria due to the loss of *fliC* transcription but also to the reduction of expression of the *hpmA* hemolysin genes. Reintroduction of *flhA* restored cell elongation and migration and resulted in differentiation-specific hyperexpression of flagellin and hemolysin genes to the levels above those seen in the parent strain (89). This could, of course, be related to the contribution of swarmer cells to the virulence, which was studied by the use of the CBA mouse model of ascending UTI and a nonmotile, nonswarming flagellar mutant, *P. mirabilis* WPM111 (an HpmA hemolysin mutant in which the assembly of intact flagellum was affected but not the synthesis of flagella, i.e., a *hpmA flaD* mutant). Results of these examinations showed that flagella themselves or the swarming differentiation which requires the synthesis of these cell surface structures contributes significantly to the virulence of *P. mirabilis* (179).

Besides the questions concerning the signal molecules which initiate cell differentiation and migration, the mechanism of overproduction of flagella by swarmer cells and the significance of this phenomenon in pathogenicity are also closer to being understood. Belas and Flaherty (26) have shown that the synthesis of swimmer and swarmer cell flagella is encoded by the genes *flaA*, *flaB*, *flaC*, and *flaD*. *flaA* and *flaB* are tandemly linked and are nearly identical copies of flagellin-determining genes, *flaC* is located an undetermined distance downstream from *flaB*, and *flaD* is responsible for flagellin assembly. It has been found that *flaA* alone, but not *flaB* and *flaC*, is expressed in wild-type *P. mirabilis*. Thus, *flaB* and *flaC* are silent copies of flagellin-encoding genes. Moreover, it has also been demonstrated that during differentiation of swimmers to swarmers, *fla* transcription increases eightfold and *flaA* is required for the induction of swarmer cell differentiation, since mutations in this region of DNA resulting in the loss of FlaA completely abolished this process.

Another aspect of this problem is associated with the important role of flagellin as a bacterial surface antigen (H antigen). Since flagellin is strongly immunogenic, it can be assumed that at least part of the immunoresponse of the host during the infection is directed against this antigen. Thus, the possible changes in flagellin antigenicity may enable bacteria to escape the immunoresponse of the infected macroorganisms. Belas (22) showed that *P. mirabilis* flagellin variation can be demonstrated in vitro (Fig. 3). By insertion of the *cam* gene, he disrupted the *flaA* reading frame and prevented the expression of *flaA*, which resulted in FlaA$^-$ mutants. However, it appeared that the spontaneous mutation within *flaA* can lead to removal not only of the *cam* gene insertion but also of the 3′ end of *flaA* and sequence in the *flaB-flaC* region. Such deletion can be followed by genetic fusion of the 5′-end region of *flaA* with the 3′-end region of previously silent copies of either *flaB* or *flaC*, which leads to the synthesis of hybrid flagellum (FlaR [Fig. 3]) containing the N-terminal portion of FlaA and the C-terminal part of FlaB or FlaC. This process enables bacteria to produce flagella antigenically distinct from those encoded by *flaA*. Indeed, Belas (22) has demonstrated that FlaR is larger

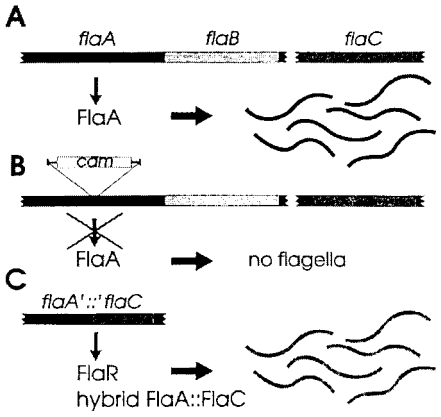


FIG. 3. Schematic model of antigenic variation *P. mirabilis* flagellin (22). (A) The *flaA* locus consists of three copies of flagellin-encoding genes; two of them, *flaA* and *flaB*, are physically adjacent to one to another on the bacterial chromosome, whereas the third is located at an undetermined distance downstream from *flaB*. *flaB* and *flaC* are silent copies of *fla* genes. Transcription of *flaA* in the wild-type strain results in the synthesis of flagellin built up from FlaA. (B) Introduction of the *cam* gene into the *flaA* locus disrupts the *flaA* reading frame leading to the FlaA$^-$ cells. (C) Spontaneous mutations within the *flaA* gene remove the *cam* gene insertion together with the 3′ end of *flaA* and part of the 5′ end in the *flaB-flaC* region. The genetic fusion of the 5′ region of *flaA* and the 3′ region of either *flaB* or *flaC* followed some deletions in this part of chromosome, resulting in the synthesis of hybrid FlaR flagellin antigenically different from FlaA. Reprinted from reference 22 with permission.

(FlaR, 40.3 kDa; FlaA, 39.4 kDa), that FlaA antiserum has limited cross-reactivity with FlaR, showing that this flagellum contains only part of the FlaA epitopes, and that *flaA* Mot$^+$ revertants are able to move through the semisolid agar containing anti-FlaA polyvalent antiserum. The last observation is in contrast to what happens when anti-FlaA antibodies are added to cultures of a wild-type *P. mirabilis* strain. In this case, bacteria cannot move, because their swimming or swarming motility is inhibited by tethering of flagella due to binding of antibodies. There is still an open question if such flagellin antigenic variation described in vitro can occur in vivo during the infection caused by *P. mirabilis*. In this context, Belas (22) points out that such antigenic changes could potentially increase the survival of *Proteus* rods colonizing the urinary tract. This phenomenon can possibly protect bacteria against the action of secreted IgA during the invasion of the bladder. It is very likely that IgA antibodies are directed against bacterial cell surface antigens, e.g., flagella. Binding of flagella by such antibodies results in tethering of bacteria and inhibition of their movement in host organisms. A spontaneous deletion, as described above, could allow bacteria to produce antigenically distinct flagella and escape their immobilization by antibodies.

Outer membrane proteins. In general, OM proteins (OMP) possess immunogenic properties and mitogenic activity for B cells (31, 49, 175, 279); furthermore, OM lipoproteins and their synthetic analogs function as adjuvants (32) and can also activate macrophages to produce tumor necrosis factor (TNF) (101). The OM of *P. mirabilis* contains three major proteins of 39.0, 36.0, and 17.0 kDa (42, 200). These proteins correspond to the ones previously shown by Hasin et al. (92), Datta et al. (63), and Lugtenberg et al. (158) to be OMP of *Proteus* spp. Hofstra et al. (102–104) showed that OMP from *P. mirabilis*, *P. vulgaris*, and *Providencia stuartii* demonstrate antigenic relationship with OMP of the other members of the *Enterobacteriaceae*. The 39-kDa protein was identified as the OmpA protein (127), and the 36-kDa protein appeared to be a peptidoglycan-associated matrix protein (158).

The OMP of *P. mirabilis* were able to mediate the penetration of hydrophilic molecules through the artificial model membranes and protect them from disaggregation by detergents (200). In particular, OmpA is effective as an immunomodulator of the immune response to LPS, greatly enhancing the level of O-specific IgG (125, 127, 199). This protein is also a mitogen for murine B cells in vitro and displays strong adjuvant activity. These three properties of the *P. mirabilis* OmpA protein could indicate that it belongs to the proteosome class of adjuvants (157). The 39-kDa protein inhibits oxygen radicals, as well as interleukin-1 (IL-1) production, and enhances TNF secretion by LPS-stimulated macrophages (306, 307). Moayeri et al. (178) have studied the ability of *P. mirabilis* K7 OMP and LPS vaccines to prevent experimental pyelonephritis in the mouse BALB/c model. They have shown that mice with *P. mirabilis* pyelonephritis produce IgG antibodies against OMP. The possible protecting effects of OMP and LPS were investigated by administering these proteins intramuscularly in Freund's complete adjuvant. After 2 weeks, the mice were challenged with bacteria of homologous K7 and heterologous *P. mirabilis* strains. It was found that mice immunized with OMP were protected from renal colonization and invasion and death by both homologous and heterologous strains whereas animals immunized with LPS were not protected from pyelonephritis. The negative results in the case of LPS were not related to specific antibody levels in vaccinated mice. The results of structural, immunochemical, and biological studies on *Proteus* LPS are presented in the next section.

Lipopolysaccharide (O-antigen, endotoxin). *Proteus* is an antigenically heterogeneous genus, principally because of structural differences of its O-specific polysaccharide chain of LPS (O antigen), as well as its H antigen. The serological classification scheme of Kauffman and Perch includes 49 different *P. mirabilis* and *P. vulgaris* O serogroups and 19 serologically distinct H antigens (128, 139, 213). Also, Penner and Hennessy (211) have introduced a separate O-grouping scheme to classify clinical isolates of *P. vulgaris* and *P. mirabilis*. In spite of these efforts, a number of S forms remains unclassified, including strains of *P. penneri* (151, 317). Serological studies of 69 *P. penneri* clinical isolates showed that only nine strains could be classified into a definite serogroup (317).

The chemical classification of *P. mirabilis* and *P. vulgaris* LPS into 16 chemotypes (262) and *P. penneri* into 7 chemotypes (317) also remains to be completed. Results of immunochemical and structural studies on all three regions of the *P. mirabilis* and *P. vulgaris* LPS O-specific part, core, and lipid A have been reviewed by Kotełko (139) and Knirel et al. (134). The composition and structure of 17 O-specific polysaccharides of *P. mirabilis*, *P. vulgaris*, and *P. penneri* strains were reported (33, 45, 46, 116, 130, 131, 133, 134, 214, 265–267, 288, 293–298). The common structural feature of *Proteus* O antigens is the presence of uronic acids sometimes substituted by amino acids. Besides the typical sugar constituents widespread in nature, like hexoses, hexosoamines, and uronic acids, they also contain 6-deoxyamino sugars like L-fucosamine, L-quinovosamine, D-quinovoso-3-amine, and D-fucoso-3-amine. From various nonsugar constituents, amino acids (L- and D-alanine, L-serine, L-threonine, and L-lysine) were attached to the carboxyl group of uronic acids. In *Proteus* O antigens, other unusual acidic components like (*R*)- and (*S*)-lactic acid ethers and (*R*)-hydroxybutyryl, pyruvic, and phosphate groups were found. In Table 4, selected *Proteus* O-antigen structures are presented.

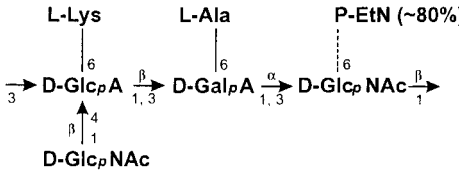
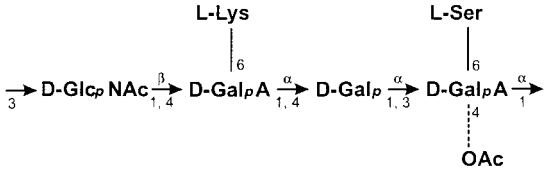
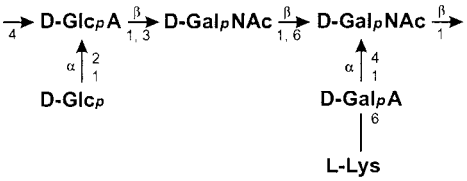
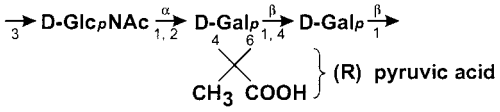
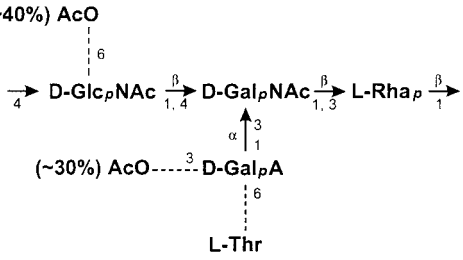
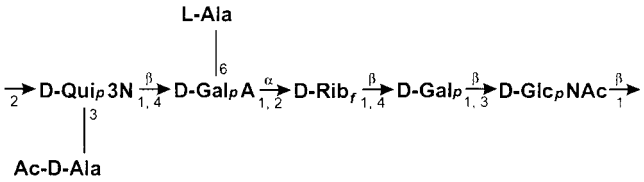
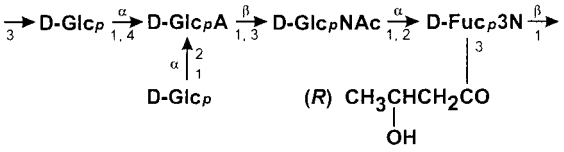
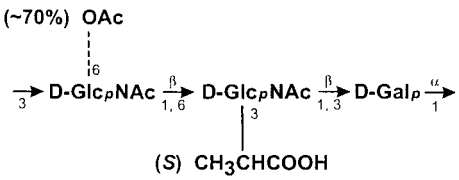
By use of polyclonal rabbit antisera, the degraded polysaccharides and their partial structures, and the synthetic antigens (51, 52) corresponding to *Proteus* O antigens, we investigated the epitopes that play an important role in their specificity. It is worth noting that the unusual LPS components mentioned above do not always play an immunodominant role. The immunodeterminant oligosaccharide characteristic for *P. mirabilis* S1959 (OXK serogroup) was found to be D-galacturonyl-1,4-D-galactosamine disaccharide substituted by lysine (85, 86, 116, 117). The immunodominant role of the lateral *N*-acetyl-D-glucosamine linked to the β-D-GlcA-L-Lys, as well as phosphoethanolamine linked to the additional residue of β-D-GlcNAc in the O-specific polysaccharide from *P. mirabilis* O27 (Table 4), was also described (146, 296). Serological studies with the synthetic antigens showed the importance of α-D-GalA-(L-Lys) and α-D-GalA-(L-Thr) in the specificity of *P. mirabilis* O28 (223) and *P. penneri* 12 (264), respectively (Table 4).

Special attention was paid to O-specific polysaccharides from *P. vulgaris* OX19 and OX2, as well as *P. mirabilis* OXK, since strains belonging to these serogroups cross-react with antibodies from patients with different rickettsial infections and are commonly used in the diagnostic Weil-Felix test (8, 9, 177, 226, 294). It was shown that LPS from spotted fever group rickettsial strains (Thai tick typhus and Katayama strains) had some components in common with LPS from *P. vulgaris* OX2, e.g., Glc, GlcNAc, and QuiNAc (quinovosamine). By use of an enzyme-linked immunosorbent assay, it was demonstrated that sera from 10 patients with Japanese spotted fever reacted with the LPS of Katayama strains and LPS from *P. vulgaris* OX2. This strongly suggested that the antigen common to spotted fever group rickettsiae and *P. vulgaris* OX2 is LPS (7).

The core region of *Proteus mirabilis* LPS was studied by using R mutants of different chemotypes, synthesizing LPS without O-specific repeating units (141), or by performing chemical analyses of core oligosaccharides isolated from S form LPS of *P. mirabilis* and *P. vulgaris* (221). Six types of core regions have been identified so far in *Proteus* LPS (224) (Table 5). However, the structures of core type II chemotype Ra (142, 222), Rc (219, 220), and Re (262, 299) have now been established (Fig. 4). The characteristic feature which distinguishes the *Proteus* core region from *E. coli* and *Salmonella* cores is the presence of D-galacturonic acid, which, in the case of core types I to III, was found both in terminal and in chain-linked positions. The terminal and chain-linked units of D-galacturonic acid were also identified in the closely related species *Providencia rettgeri* and *M. morganii*, as well as in *S. marcescens*, which demonstrated some similarities to *Proteus* spp. (140, 221). The presence of uronic acid in the core region of *K. pneumoniae* R20/O1 was shown by Süsskind et al. (280). Intensive structural investigations performed by Sidorczyk et al. (261, 268) and Vinogradov et al. (299) led to the full structure of *P. mirabilis* R45 LPS (Re chemotype), containing lipid A and 3-deoxy-D-manno-octulosonic acid (Kdo) disaccharide substituted by AraN (Fig. 4). Chemical and structural analysis of LPS from *P. mirabilis* R14/1959 showed that this mutant synthesized high-molecular-weight polysaccharide differing in its composition from the repeating units of the parental strain *P. mirabilis* S1959, linked to the core oligosaccharide. Because of some similarities to the chain in *Salmonella friedenau* T1 LPS, the name "T chain" for this high-molecular-weight part of LPS R14/1959 was proposed. Methylation analysis and nuclear magnetic resonance spectroscopy studies revealed that T antigen from *P. mirabilis* R14/1959 was identical to O antigen from *P. penneri* 42 (18, 19, 132).

The knowledge of the chemical structure of the core region of *Proteus* LPS led us to initiate studies on the epitope specificity of antisera against *P. mirabilis* R mutants. We found that galacturonic acid plays an important role in the specificity of *P. mirabilis* R110 LPS, as well as T antigen from *P. mirabilis* R14

TABLE 4. Structures of O-specific polysaccharides from some *Proteus* lipopolysaccharides

O serogroup or strain	Structure of repeating unit	Reference
P. mirabilis		
O27		296
O28		223
1959		116
P. vulgaris O19		214
P. penneri		
12		264
14		297
16		298
62		131

(18). It was also demonstrated that the majority of antibodies present in *P. mirabilis* R45 antiserum recognized an epitope which comprised both the Kdo and lipid A domains. Anti-Kdo or anti-lipid A antibodies were not found in this antiserum (237).

Figure 5 shows the structure of *P. mirabilis* lipid A (263, 268). It differs from lipid A of *E. coli* and *Salmonella minnesota* in the composition of fatty acids and the presence of 4-amino-4-deoxy-L-arabinosyl residue, which quantitatively substitutes the ester-linked phosphate residue of its glucosamine back-

TABLE 5. Core types of *Proteus* LPS[a]

Core type	LPS	Constituents								
		Common					Characteristic			
		GalA	LD-Hep	Kdo	EtN	Glc	DD-Hep	GlcN	GalN	Gal
I	*P. mirabilis* O28, O23, O32; *P. vulgaris* O39	●	●	●	●	●		○		
II	*P. mirabilis* 1959	●	●	●	●	●	○	○		
III	*P. mirabilis* O28	●	●	●	●	●	○		○	
IV	*P. mirabilis* O38	●	●	●	●	●			○	○
V	*P. vulgaris* O23	●	●	●	●	●	○		○	○
VI	*P. mirabilis* O11, O13, O30, O31, O35, O36	●	●	●	●	●	○	○	○	○

[a] For details, see reference 224.

bone. Based on the result of chemical analysis of lipid A from *P. mirabilis* R45 mutant, *Proteus* lipid A was synthesized and some of its biological effects (mitogenic activity, lethal toxicity, and the local Shwartzman reaction) were found to be comparable to those of *E. coli* and *Salmonella* synthetic analogs (260).

Biologically, LPS are endotoxins, well-known pathogenic factors of gram-negative bacteria, which cause a broad spectrum of pathophysiological effects such as fever, hypotension, disseminated intravascular coagulation, and lethal shock (231). Endotoxin can be released from cell surfaces of bacteria during their multiplication, lysis, and death. Such a free LPS is a bioactive molecule and acts through its biological center (lipid A component) on various cell types, of which macrophages and monocytes are the most important. We already understand the mechanisms of biological activity of LPS. It binds to the LPS binding protein in the blood; this complex then activates a CD14 receptor on macrophages. LPS-induced activation of these cells results in the production of biologically active lipids (prostaglandins, thromboxane A_2, and platelet-activating factor), oxygen free radicals (O_2^-, H_2O_2, and NO), and peptide mediators (TNF-α, IL-1, IL-6, IL-8, and IL-10). These mediators act independently or in concert, and, depending on their level in the macroorganisms, they elicit beneficial (e.g., adjuvant activity) or detrimental (e.g., shock syndrome) effects (229). On the other hand, LPS as a bacterial surface antigen is recognized by specific antibodies produced by the host defense system. LPS from the S form of pathogenic bacteria contributes to their resistance against bactericidal action of serum and intracellular killing by phagocytes (230).

The biological properties of endotoxin described above also refer to *Proteus* LPS. It was demonstrated that LPS from the S and R forms of *Proteus* showed inhibitory activity toward mouse liver cytochrome P-450 (118). It is also worth noting that human hemoglobin decreases complement fixation by different *Proteus* LPS (120). In contrast, some other biological effects of *Proteus* LPS were increased by the addition of human

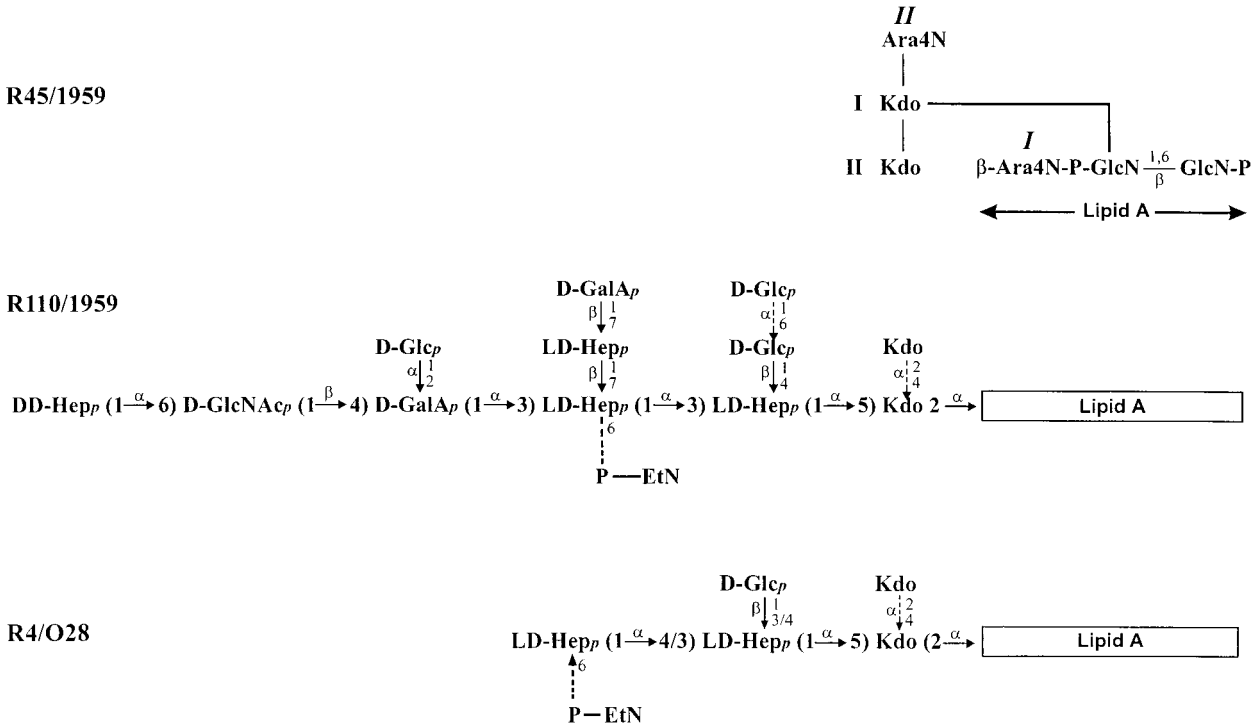


FIG. 4. Structure of core oligosaccharides of *P. mirabilis* LPS (219, 222, 268, 299).

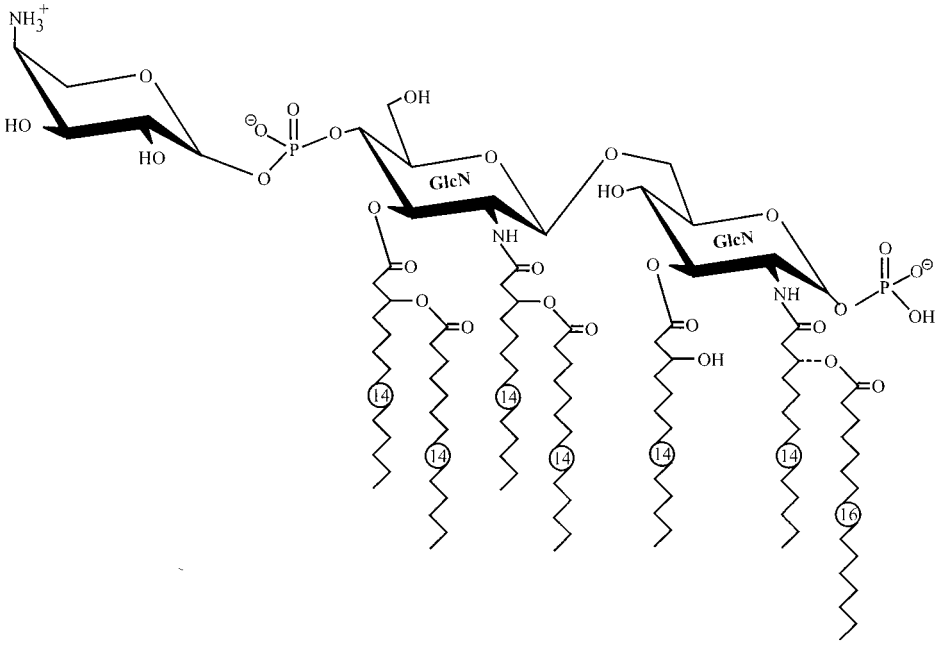


FIG. 5. Chemical structure of the lipid A component of *Proteus* LPS (268).

hemoglobin, as measured in the *Limulus* assay (121, 122). The chain length of LPS plays an important role in its interaction with the bactericidal/permeability-increasing protein in such a way that long polysaccharide chains inhibit this interaction (43). *Proteus* infections may result in bacteremia and septic shock (166). Epidemiological investigations showed that among *Proteus* spp., strains classified into the O3, O27, O10, and O28 serogroups occurred most often in clinical isolates (151). However, this observation was not correlated with the resistance of these strains to the bactericidal effect of antiserum (115). It was found that *Proteus*, *Morganella*, and *Providencia* strains isolated from urine of patients with UTI were more frequently resistant to the action of normal human serum than were strains isolated from feces (195). The role of *Proteus* strains in glycocalyx formation was described previously (56) and is discussed below. Larsson and Olling (153) reported on the isolation of spontaneously agglutinating phenotypically R-like forms from the urine of patients with chronic UTI. One of these strains—*P. mirabilis* 17301, which synthesized LPS of the Rc chemotype—was shown to survive in the kidneys when tested in a hematogenous infection model in mice. *P. mirabilis* 17301 was characterized as the most invasive compared to the other *Proteus* S forms and R mutants (148). The data mentioned above indicate that *P. mirabilis* strains may be pathogenic even if they synthesize a very incomplete LPS. The role of endotoxin in UTI is not clear at this time. The possibility of development of local nephritis in dogs was demonstrated after direct injection of lipid A into the renal pelvis. Such administration of lipid A results in an immunopathophysiological process in which complement activated by lipid A–anti-lipid A complexes causes inflammation (312).

Capsule antigens. For a long time, it was believed that *Proteus* strains did not produce the typical capsule antigens characteristic of some gram-negative bacteria, e.g., *Klebsiella* spp. or particular *E. coli* strains. The capsule structure, also termed as slime material or glycocalyx (highly hydrated polymers present on the surface of bacteria), was demonstrated to be a potential pathogenic factor of *Proteus* strains because of its positive effect on struvite crystal growth and stone formation (33, 68). Detailed studies showed that *P. mirabilis* O6 and O57 and *P. vulgaris* O19 could synthesize a capsule antigen structure identical to the O-specific chain of their LPS (33, 214, 288). The acidic character of *Proteus* CPS, due to the presence of uronic acids, pyruvic acid, or phosphate groups, enabled them to bind metal cations (e.g., Mg^{2+}) via electrostatic interactions. Dumanski et al. (68) compared struvite growth in vitro in urine in the presence of purified CPS of known structure and varying in charge. It was found that of the tested capsule antigens, CPS of *P. mirabilis* ATCC 49965 enhances the formation of struvite crystals. This effect was correlated with the low affinity of *P. mirabilis* CPS for Mg^{2+}. The authors have speculated that the weak binding of Mg^{2+} by this CPS facilitates the growth of struvite crystals, because magnesium ions being accumulated can be readily released from LPS for calculus formation (68). Other aspects of CPS formation by *P. mirabilis* rods are reported in the section on flagella and swarming motility, above.

Natural Resistance of *Proteus* Rods to Polymyxins

Proteus spp. are naturally resistant to polycationic, cyclic antibiotics, i.e., polymyxins. These antibiotics, due to the positive charges in the structure, bind negatively charged surfaces of bacterial cell envelopes, e.g., LPS, capsule antigens, and phospholipids. This results in a disorganization of the outer and inner membranes of gram-negative bacteria (290, 292). Polymyxin B (PmxB) is a good inhibitor of LPS-induced synthesis of TNF-α by macrophages and release of IL-1 from human macrophages (44, 62). Moreover, PmxB interacts with negatively charged Kdo-lipid A region of LPS (189, 244). The relationship between the composition of LPS and the ability to bind polycationic antibiotics by *Enterobacteriaceae* was demonstrated by Vaara et al. (291), who showed that complete substitution of the ester-linked phosphate group of lipid A by L-arabinoso-4-amine (in *S. typhimurium pmrA* mutants) led to

the resistance of bacteria to PmxB action. This also explains the natural resistance to this antibiotic of several *P. mirabilis*, *M. morganii*, *Providencia rettgeri*, and *Serratia marcescens* strains, since LPS of these bacteria contain L-arabinoso-4-amine (20). In LPS of *P. mirabilis* R45 (mutant Re), this compound not only is present in lipid A but also is attached to the Kdo residue by a (1 → 8) linkage (Fig. 4) (261, 299). Moreover, it was shown that *P. mirabilis* mutant R4 (Rc), which lacked arabinoso-4-amine residues, appeared to be sensitive to PmxB activity (119). Recently Boll et al. (35) have demonstrated that the L-Ara*p*4N present in the inner core region of LPS (attached to Kdo residue) can play a decisive role in the decreasing of binding of PmxB, which results in the resistance of bacteria to this antibiotic.

Invasiveness

Cell invasiveness, also termed cell penetration, is an important step in infection and has been investigated, in relation to *Proteus* rods, by several groups of authors (75, 76, 207, 238). Braude and Siemienski (36) found that the invasion of mammalian cells by *P. mirabilis* rods in vivo and in vitro was stimulated by urea. It was shown by others that all *P. mirabilis* and *P. vulgaris* strains investigated, including those from patients with UTI as well as those from healthy persons, were able to penetrate Vero cells (the African green monkey kidney cells) (207, 208). A correlation of cell-associated hemolytic activity with penetration was also observed (207). A statistically higher Vero cell invasiveness by strongly hemolytic strains of *P. mirabilis*, as compared with *P. vulgaris* strains with low hemolytic activity, was found (208).

The penetration ability of *P. mirabilis*, *P. vulgaris*, and *P. penneri* strains has also been studied by our group (148, 238, 239). We have shown that all the cell lines used (Vero, HeLa, L-929 mouse fibroblasts, and human blood lymphocytes) were penetrated by these bacteria. The maximal invasiveness was observed between 3 and 5 h after incubation of the tested cells with bacteria. The invasiveness was markedly higher for *P. mirabilis* strains producing cell-associated hemolysin (238, 239), which was in agreement with data obtained by Peerbooms et al. (207, 208). In the course of a prolonged observation time, survival and subsequent multiplication of *P. mirabilis* rods in invaded cells were noted, not only in the stable L-929 cell line but also in fresh human blood lymphocytes (207). Of the *Proteus* S forms and R mutants investigated in our laboratory, *P. mirabilis* 17301 was the most invasive and colonized the L-929 mouse fibroblasts in a very short time (148). This strain was isolated from the urine of a patient with asymptomatic bacteriuria (153) and was a phenotypically rough form similar to the *P. mirabilis* mutant class Rc chemotype (147, 148). Its penetration ability was higher than that of *P. mirabilis* S forms, which suggested that the hydrophobicity of its cell wall can contribute to the invasiveness phenomenon. It cannot be excluded either that *P. mirabilis* 17301 synthesized a protein invasiveness factor(s), like *Yersinia pseudotuberculosis* invasin (107) or *Listeria monocytogenes* internalin (79). Such an invasion-related surface protein has not been identified on *Proteus* rods so far. However, Moayeri et al. (178) have found that OMP from *P. mirabilis* could be useful as antigens to raise antibodies preventing pyelonephritis in the BALB/c mouse model.

The ability of *P. penneri* strains producing cell-free HlyA hemolysin to penetrate Vero and L-929 cell lines was significantly different from that of *P. mirabilis* and *P. penneri* strains synthesizing cell-associated HpmA hemolysin (239). Maximal penetration of *P. penneri* strains not producing HlyA hemolysin occurred at 2 h and was not accompanied by a cytotoxic effect. The penetration of Vero cells by HlyA *P. penneri* strains was markedly different; bacterial invasion was observed until 3 h, and after that it was dramatically decreased, due to the cytotoxic effect of extracellular hemolysin (239). These results were confirmed in separate studies which compared the abilities of *P. penneri* $HlyA^+$, $HpmA^+$ and $HlyA^+$ $HpmA^+$ strains to penetrate the mouse L-929 fibroblasts (236). Strains synthesizing HlyA cytolysin were strongly cytotoxic, in contrast to strains expressing HpmA hemolytic activity, which evoked a weak cytotoxicity. The results of these studies showed that hemolytic *P. penneri* strains were able to penetrate tested cells; however, the cytotoxic activity of HlyA hemolysin produced by them destroyed the L-929 fibroblast monolayer. It is not clear if this destructive effect was due to the action of hemolysin from outside the monolayer or to the cytolysin released from bacteria which had been previously internalized. These bacteria were killed by the gentamicin used in this assay. Such observations have been made recently by Chippendale et al. (54), who studied the internalization process of *P. mirabilis* strains by human renal epithelial cells. For this investigation, confluent monolayers of primary cultures of human renal proximal tubular epithelial cells (HRPTEC) were used. It was demonstrated by light and electron microscopy that internalized bacteria were present within membrane-bound vacuoles of kidney cells. Cytochalasin D does not inhibit this process, suggesting that microfilament formation due to the actin polymerization is not involved in the mechanism of internalization of *P. mirabilis* bacilli by HRPTEC. Moreover, it was shown that the nonhemolytic *P. mirabilis* WPM 111, which was not able to disrupt the monolayer, was 10- to 100-fold more invasive than a hemolytic parent strain, BA 6163. Chippendale et al. (54) also found that short rod-shaped vegetative forms of *P. mirabilis* internalized within membrane-bound vacuoles were not able to replicate, which was in contrast to the results obtained by Allison et al. (3) and Różalski et al. (238). These discrepancies between all the studies may be due to the different cell cultures used.

As mentioned above, Allison et al. (3) also showed that the ability to invade Vero cells and two human uroepithelial cell lines, EJ 128 and 5637, was closely coupled to the swarming phenomenon. It was shown that invasion by swarmer cells occurred within 30 min and was about 15- to 20-fold greater after 2 h when compared with invasion by vegetative cells, which were internalized more slowly. The cell penetration by swarmers was complemented by overproduction of proteases, urease, and cell surface-bound hemolysin (HpmA) (3). By use of different transposon (Tn*5*) mutants with specific defects in motility and multicellular behavior, it was possible to study the role of the swarming cycle in the invasion process. The nonflagellated mutants, which were nonmotile and nonswarming, were completely noninvasive. Mutants which were motile but defective in swarmer formation (motile, nonswarming mutants) had very limited invasion ability. Similar results were obtained when using mutants with defects in coordination of multicellular migration and control of consolidation. The addition of flagellin to the environment did not significantly influence the invasiveness of any of the investigated mutants. In this study, it was also shown that nonhemolytic mutants were only slightly less able to invade tested cells than was the wild-type form. This is in contrast to the above-mentioned results of Peerbooms et al. (207) and Różalski et al. (238). The data obtained by Allison et al. (3) indicated that differentiated swarmer cell filaments of *P. mirabilis* were the most invasive forms of these bacteria and may play a major role in the colonization of human epithelium during UTI. The mecha-

TABLE 6. Comparison of *ure* clusters and *ure*-encoded polypeptides of *P. mirabilis*, *P. vulgaris*, and other selected gram-negative bacteria[a]

Species	*ure* genes sequenced	Predicted mol mass of *ure*-encoded polypeptides (kDa)							
		A	B	C	D	E	F	G	R
P. mirabilis	RDABCEFG	11.0	12.2	61.0	31.0	17.9	23.0	22.4	33.4
K. aerogenes	DABCEFG	11.1	11.7	60.3	29.8	17.6	25.2	21.9	
Y. enterocolitica	ABCEFGD	11.1, 11.3	17.9	61.0, 61.5	36.4	29.5	25.0	24.1	
P. vulgaris	ABC	11.0	12.1	61.0					
K. pneumoniae	DA	11.1			30.0				

[a] For details, see reference 184.

nisms of this process are not clear. High activity in swarmer cells of two important virulence factors, HpmA hemolysin and urease (due to specific transcriptional activation) and possibly synthesis of invasins (cell surface proteins [not yet proved]) are thought to be crucial to the invasion process and thereby to the pathogenicity of *Proteus* rods. Until now, the results of studies of Allison et al. (3) have not been confirmed by other laboratories. Chippendale et al. (54), who also studied the penetration of *P. mirabilis* swarmer cells by HPRTEC, found that when added to monolayers, these cells rapidly reverted to short rods and then were internalized in this form. A similar observation was made in our laboratory when testing the invasiveness of *P. mirabilis* swarmer cells for L-929 mouse fibroblasts (235).

Urease

Urea represents the main nitrogenous excretory product in humans and the majority of animals. Urease (urea amidohydrolase; EC 5.1.5) catalyzes the hydrolysis of this compound to yield ammonia and carbon dioxide, which results in an increase in the urine pH (56, 172, 184, 185). Urease activity has been found in over 200 species of gram-negative and gram-positive bacteria (84, 172, 180, 185). This enzyme has also been implicated as a factor contributing to the pathogenicity of many bacteria including *Proteus*, *Providencia*, and *Morganella* species (88, 112, 164, 165, 212, 234, 251). The urease activity of these bacteria is used to distinguish them from other *Enterobacteriaceae* family members. This activity was constitutive in most *P. mirabilis* strains (184), plasmid mediated (e.g., *Providencia stuartii*) (172, 184), and inducible (in some *P. mirabilis*, *P. vulgaris*, and *P. penneri* strains [187], as well as in *Providencia rettgeri* [184]). The unique feature, characteristic of bacterial ureases, is their association with nickel and its large number of cystine residues (11, 181, 225, 275).

In cell fractionation studies, it was shown that the majority of urease from *P. mirabilis* is present in the soluble cytoplasmic fraction (113). The opposite results were obtained when electron microscopic methods were used; it was found that *P. mirabilis* urease was associated with the periplasm and OM (171). However it is important to note that detection was done by monitoring the product of hydrolysis, that is, ammonia but not urease itself. *P. mirabilis* urease in its native form is a 212- to 280-kDa protein containing α, β, and γ subunits (38, 112, 184). *P. mirabilis* urease was stable at 0°C in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) in buffer containing 1 mM EDTA and 1 mM 2-mercaptoethanol (pH 7.5). It was also stable for 24 h at pH 7 to 10 at 0°C (184), whereas at 37°C the stability was observed only in the range of pH 8 to 9 (38). At pH 7.5, *P. mirabilis* urease was stable for 10 min at 60°C and completely inactivated at higher temperatures (184). The K_m value of this enzyme varied from 13 to 60 mM urea (38, 112). The activity of *Proteus* ureases can be inhibited by several substances, e.g., structural analogs of urea (hydroxyurea, thiourea, and methylurea), acetohydroxamic and hippuric acids, phosphoramides, and boric and boronic acids (38, 184). An increase in the urease activity was caused by posttranslational insertion of nickel into performed apourease (225).

The operon of *P. mirabilis* urease (6.45 kb) shows homology to the urease operon of the related species *Providencia stuartii* (113, 186, 301). The sequence of the *P. mirabilis* urease operon shows that it contains seven open reading frames, *ureA* to *ureG* (Table 6). The proteins determined by *ureA*, *ureB*, and *ureC* are α (UreA), β (UreB), and γ (UreC) structural subunits of urease with predicted molecular masses of 11.0, 12.2 and 61.0 kDa, respectively. The additional polypeptides, UreD, UreE, and UreF (31.0, 17.9, and 23.0 kDa, respectively), were encoded by accessory genes and were involved in nickel ion insertion into the apoenzyme (114). For the full enzymatic activity of *P. mirabilis*, urease polypeptide UreG (22.4 kDa), determined by sequence downstream from *ureF* (Table 6), was also required (276). Disruption of *ureD*, *ureF*, or *ureG* led to production of inactive enzyme without nickel ions, whereas disruption of *ureE* reduced only the level of urease activity. It is speculated that UreD plays the role of a urease-specific chaperone protein important for the formation of active enzyme (180).

Transcription of the enzyme structural genes *ureA*, *ureB*, and *ureC* is repressed by a *trans*-acting protein encoded by *ureR*. It was found that derepression of transcription, probably due to the binding of urea to *ureR*, led to the expression of structural genes which determined the synthesis of urease (197). Recently, it has been found that *ureR* is transcribed from its own promoter in a direction opposite to that of the transcription of the rest of the operon. The UreR is predicted to be a 33.4-kDa protein, characteristic for the DNA-binding regulatory protein family, with 18 Glu and 16 Lys residues, which probably take part in the binding of the DNA sequence. It is probable that this protein contains a helix turn which binds the helix DNA-binding domain. This part of UreR is similar to the DNA-binding proteins (AraC family, regulating acid phosphatase synthesis [AppY], porin synthesis [EnvY], and rhamnose utilization [RhaR]) (191). UreR activates the transcription of urease structural and accessory genes *ureD* and *ureA* from a promoter upstream of *ureD* (108, 190).

The role of urease in infections has been studied by several authors (36, 81, 162, 163, 194), and this enzyme has been demonstrated to be a significant virulence factor in *P. mirabilis* strains. Mobley et al. (182) have shown in vitro in cultured human renal proximal tubular epithelial cells that its cytotoxic effect is of minor importance in comparison with that of HpmA hemolysin. The constructed urease-negative mutant of *P. mirabilis* containing an insertion mutation within *ureC* was used to demonstrate the significant role of urease in a mouse model of ascending UTI (109, 111). Particularly, it was found that the urease-negative mutant had a 50% infective dose (ID_{50}) over 1,000-fold greater than that of the parent strain. This mutant

was cleared from the bladder, whereas the urease-positive strain persisted in the bladder and kidney and caused significantly more severe renal lesions.

P. mirabilis and *P. penneri* are the most common microorganisms implicated in kidney and bladder stone formation (144, 233). Urease plays the major role in this phenomenon (56, 172, 184). It is well known that hydrolysis of urea leads to elevation of the urine pH, which results in precipitation of urinary components such as Mg^{2+} and Ca^{2+}, which are soluble at the slightly acidic or neutral pH characteristic in normal urine. As a result of this effect, struvite ($MgNH_4PO_4 \cdot 6H_2O$) or carbonate apatite [$C_{10}(PO_4)_6 \cdot CO_3$] stones or both are formed (56, 172, 184). This phenomenon does not take place during UTI due to urease-negative *E. coli* (184).

It is also known that in addition to urease activity, the organic gel-like surrounding contributes to the stone formation mechanism. This organic medium, also called a capsule or glycocalyx, consists of exopolysaccharide, which facilitates the adhesion of bacterial cells to the tissue surface. Bacteria can divide in the glycocalyx, which finally leads them to form isolated clumps or microcolonies as well as continuous biofilm (50, 56, 172). Biofilm formation is common not only to the urinary tract but also to the other tissues, artificial surfaces (e.g., catheters), and natural and industrial water systems. It protects bacteria against antimicrobial agents and leukocytes, and facilitates the binding of metal ions from the surroundings. In light of the knowledge of bacterial glycocalyx and biofilm formation, Clapham et al. (56), in in vitro studies, revealed a strong influence of bacterial capsule on the crystal growth of stones. From this experiment, it can be concluded that struvite crystals may grow within *P. mirabilis* biofilms. Taking this into account, McLean et al. (172) have formed an etiological concept of stone formation which can be summarized as follows: (i) *P. mirabilis* ascends the urinary tract, colonizes uroepithelium, and then forms glycocalyx-enclosed microcolonies and facilitates bacterial adhesion; (ii) urease production by bacteria elevates urine pH, resulting in the precipitation of struvite and carbonate apatite crystals, which settle down in glycocalyx, also protecting uropathogens from antibiotics, antibodies, and urease inhibitors, as well as host defense mechanisms (glycocalyx is also a good ground for incorporation of host mucoproteins and polysaccharides into the matrix); and (iii) encrusted, subdividing bacteria continue production of glycocalyx, synthesis of urease, and incorporation into the matrix elements of host tissue; in this microenvironment, nucleation and growth of struvite and carbonate apatite crystals stones occur, and finally the mature stone looks like an enlarged "fossilized" bacterial colony (56, 172, 173, 198).

IgA and IgG Proteases

IgA in the form of secretory protein (sIgA) is a dimer of IgA held together by the J chain and contains the secretory component used to transport the antibody molecule. The sIgA is the predominant Ig in mucus secretion. Its function is to protect mucous membrane and underlying tissue from bacteria and their products. sIgA is markedly resistant to degradation by proteolytic enzymes of many microorganisms examined; only a few microbes have been found to synthesize extracellular proteolytic enzymes capable of degrading IgA. These include *Neisseria meningitidis*, *N. gonorrhoeae*, *H. influenzae*, and *Streptococcus pneumoniae*, which are associated with diseases at mucosal surfaces, as well as some periodontal pathogens (192, 217). Because other related but not pathogenic species of the same bacterial genera do not synthesize IgA proteases, production of these enzymes may be associated with the virulence. Bacterial IgA proteases are distinguished from other proteolytic enzymes by their extremely narrow substrate specificity, which is restricted to the IgA1 subclass of Igs produced by humans, chimpanzees, and gorillas. IgA proteases cleave the heavy chain of the IgA1 isotype at a specific site within a 13-amino-acid proline-rich polypeptide segment in the hinge region. Since this sequence is not present in IgA2, this subclass of Ig is resistant to IgA protease action (135).

It was reported that *P. mirabilis* strains of different origins (250, 255), as well as *P. vulgaris* and *P. penneri* strains (156), produce IgA proteases. The most characteristic feature of *P. mirabilis* IgA proteases is the cleavage of Ig heavy chain outside the hinge region (249). It was also found that about 50% of the investigated *P. vulgaris* strains exhibit this activity. However, the frequency of IgA protease production by strains belonging to biotypes 2 and 3 was different (250). A *P. mirabilis* protease which hydrolyzed myeloma IgA to smaller fragments was also reported (176).

An extracellular proteolytic enzyme which cleaves two classes of antibodies, IgA and IgG, as well as non-Ig proteins such as gelatin, secretory component, casein, and bovine serum albumin, was isolated from a strain of *P. mirabilis* isolated from patients with chronic UTI (155). The enzymatic activity was demonstrated to be due to a 50-kDa polypeptide, which probably cleaved the α chain of IgA mainly between the C_H2 and C_H3 domains to produce the 47-kDa fragment, with extensive breakdown of the released C_H3 domain. Cleavage of the IgG by *P. mirabilis* protease was a two-stage process. The first step led to the elimination of $F(ab')_2$ and pFc fragments, whereas the second step generated Fab and Fc fragments (155).

Proteus proteinases are metalloenzymes that are similar in some respect to metalloproteinases of *Pseudomonas aeruginosa* and *Serratia marcescens* (156, 255). The optimum pH for their action was 8, which is not surprising because of the alkaline surroundings in which enzymes "work" in vivo (156). It seems that during infection, *P. mirabilis* strains synthesize urease, which degrades urea, resulting in the production of alkaline conditions optimal for the action of IgA (and IgG) proteases. Both types of Igs, IgA and IgG, are present in urine, in a ratio of about 1:3. Moreover, the urine of patients with *P. mirabilis* UTI contained α-chain fragments identical in size to those formed as a result of in vitro action of *Proteus* proteases. Such fragments were not detectable when the infecting strain was not proteolytic (248). Some of the urine specimens also contained proteases of the same size as the above-mentioned isolated and characterized enzymes, which provides direct evidence of the synthesis of these kinds of enzymes in vivo.

Recently, Wassif et al. (305) reported on the biochemical characterization and the genetic and sequence analysis of a recombinant extracellular metalloprotease (Zap A; 55 kDa, 488-amino-acid polypeptide) from *P. mirabilis*. This enzyme is encoded by the *zapA* structural gene (1.4 kb long), which is part of the gene cluster. It is able to digest both serum and secretory forms of IgA1 and IgA2 from both humans and mice, as well as IgG, and its activity is stimulated by the divalent cations Ca^{2+} and Mg^{2+}. Zap A metalloprotease is very similar to the IgA and IgG proteases of *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Erwinia chrysanthemi*. All these proteins belong to the serralysin family of zinc metalloproteases, which are members of the ABC superfamily of prokaryotic and eukaryotic transporters. The serralysin family is a branch of a larger group of virulence proteins from pathogenic *Actinobacillus* spp., *Bordetella pertussis*, enterohemorrhagic *E. coli*, *Neisseria meningitidis*, and *Pseudomonas fluorescens*.

Allison et al. (3, 4) have demonstrated that the differentiation of *P. mirabilis* short vegetative rods into filamentous,

multinucleate, and hyperflagellate swarmer cells is accompanied by substantial increases in the activities of virulence factors, including proteases. Since the ability of *P. mirabilis* to invade human urothelial cells is primarily characteristic of swarmer cells but not vegetative bacilli, the protease activity has been hypothesized to be relevant to differentiation and possibly to the pathogenicity of *P. mirabilis*.

The role of *P. mirabilis* IgA and IgG proteases as virulence factors in UTI seems to be important. However, the absolute amount of specific antibody isotypes in the urinary tract is not known. Since complement is not secreted in large amounts, it is possible that host defense against the infection process occurring in the urinary tract includes antibody-mediated opsonization of bacteria and, afterward, binding of the Fc fragment of antibodies to phagocytic cells. This defense mechanism may play a major role, since IgA and IgG antibodies can be opsonic and the Fc receptor of IgA has been identified on human polymorphonuclear cells. Thus, the synthesis and action of the above-described proteinases in vivo in the urinary tract might diminish or even eliminate phagocytosis due to hydrolysis of opsonic antibodies to ineffective fragments (156).

Hemolysins

The synthesis of cytotoxic hemolysins is common among both gram-negative and gram-positive bacteria. The history of investigation of *Proteus* hemolytic activity is very long and starts from the beginning the 20th century, when Wenner and Rettger (311) were not able to find this activity whereas Taylor observed lysis of erythrocytes in young broth cultures of *Proteus* bacilli (286). More detailed studies by Philips (215) showed the differences in lysis of erythrocytes of different origin by *Proteus* strains. The relationships between the production of extracellular hemolysin and virulence of *Proteus morganii*, now designated *Morganella morganii*, were described by Emödy et al. (71). Systematic studies of hemolytic activity of *P. mirabilis* and *P. vulgaris* strains were conducted in our laboratory. It was found that most of the 84 strains isolated from patients with UTI were able to degrade erythrocytes, manifested as greenish discoloration of blood agar plates. None of these strains exhibited extracellular hemolytic activity (142, 143). A similar picture of hemolysis on blood agar was described by Senior and Hughes (252), who found that 32 *P. mirabilis* strains, including isolates from patients with UTI and from feces, were nonhemolytic within 16 h at 37°C. However, after incubation, discoloration of medium was observed. Of 31 *P. vulgaris* strains representing biotypes 2 and 3 also examined under these conditions, 48% displayed hemolysis, which was visible as large, narrow or wide zones around the colonies. There was no association between the biotype of the investigated *P. vulgaris* strains and hemolysis.

In other studies, it was found that all 126 *P. mirabilis* and *P. vulgaris* strains, representing urinary isolates, laboratory collections, and isolates from soil, were able to hemolyze human and sheep erythrocytes in gently aerated cultures in nutrient broth (142, 143, 148). Moreover, 10 *P. mirabilis* strains tested under the same conditions also hemolyzed guinea pig, rabbit, mouse, horse, cattle, and hen erythrocytes. Similar results were obtained only when *Serratia marcescens* strains were used (240). This hemolytic phenomenon was shown to be cell associated and could be demonstrated only by actively growing and multiplying cells in the presence of erythrocytes. Cell-associated hemolytic activity also interested Peerbooms et al. (206, 208), who showed that these properties occurred at a significantly lower level in *P. vulgaris* strains than in *P. mirabilis* ones. Significant differences were not observed in the expression of production of cell-bound hemolysin by virulent *P. mirabilis* strains isolated from patients with pyelonephritis and catheter-associated or fecal isolates, except for fecal isolates from hospitalized children, which exhibited higher activity than did isolates from chronically catheterized elderly patients (181). Comparable results were reported by Senior and Hughes (252), who were also interested in cell-associated hemolytic activity of the family *Proteeae*. They observed that *P. mirabilis* and *P. vulgaris* strains exhibit this kind of hemolysis in detectable amounts in early log phase and that hemolysis reached its maximum in the mid-log to late log phase; thereafter, the activity rapidly declined. This phenomenon was calcium independent (252). Similar results were obtained by our group (123, 142). The production of cell-associated hemolysin by *P. mirabilis* S1959 was comparable in nutrient broth, Luria broth, and semisynthetic M9 medium supplemented with 0.4% glucose and 0.3% yeast extract. The cell-bound hemolysin was also produced in semiliquid and semisynthetic M9 medium supplemented as above, containing 0.1 to 0.5% agar. Sheep erythrocytes were lysed not only in aerated cultures but also under anaerobic conditions and in cultures with reduced redox potential achieved by adding cysteine. This hemolytic activity was observed at 28, 37, and 42°C but not at 4 or 10°C. The maximal hemolysis was expressed by bacterial cultures in the logarithmic and stationary phases of growth. The hemolytic factor seems to be strongly cell associated and not stable, since no hemolytic activity was observed in cell-free supernatants and filtrates of bacterial cultures growing under the conditions mentioned above, as well as in material after the disintegration of bacterial mass. Hemolytic activity was found in cell-free filtrates of the cultures of one strain of *P. mirabilis* and one strain of *P. vulgaris* in the death growth phase. The manifestation of this activity was considered to be a result of autolysis of bacteria in old, decaying cultures and "natural secretion" of formerly cell-bound hemolytic factor. This hemolysin was Ca^{2+} independent and negatively charged (123).

A more complex investigation of the hemolytic activity of *Proteus* rods, also from the genetic point of view, was performed by Koronakis et al. (136–138) and Welch (308). The first authors distinguished three types of hemolytic activity: intracellular and cell-associated hemolysis, characteristic of all investigated strains of *P. mirabilis*, *P. vulgaris*, and *M. morganii*, as well as cell-free hemolysis, found in *P. vulgaris* and *M. morganii* but not in *P. mirabilis* (136). The production of active intracellular hemolysin was similar in all investigated strains, although its level was considerably higher than that seen in the *E. coli* strain used as a control. The dynamics of synthesis of intracellular hemolysin were comparable in these strains; maximal activity was observed in the mid-logarithmic phase of growth and rapidly decreased as culture growth ended. Extracellular hemolysin was produced by all tested *M. morganii* strains and about 40% of *P. vulgaris* strains but none of the *P. mirabilis* strains, which synthesized cell-bound hemolysin. The studies of hybridization of DNA from hemolytic isolates of *P. mirabilis*, *M. morganii*, and *P. vulgaris* to the cloned *E. coli hly* determinant showed incomplete homology among the genes encoding production of hemolysins in all four tested strains. DNA from *P. mirabilis*, *P. vulgaris*, and *M. morganii* hybridized with *E. coli hlyA* and *hlyB* genes. One of the two *E. coli* secretion genes—*hlyD*—hybridized only with DNA from *P. vulgaris* and *M. morganii* (strains producing cell-free hemolysin) but not with DNA from *P. mirabilis* exhibiting cell-associated hemolytic activity. Moreover, the molecular cloning of the total cellular DNA from *P. vulgaris* and *M. morganii* showed the relationship between the *hly* genes of these strains and those of *E. coli*. It was also interesting that elimination of secretion of *P.*

vulgaris and *M. morganii* hemolysin by transposon mutation could be specifically complemented by addition of the *E. coli* secretion genes *hlyB* and *hlyD*. These data led the authors to the conclusion that the secreted hemolysins of *P. vulgaris* and *M. morganii* are strongly genetically related to each other and to *E. coli* alpha-hemolysin (136–138).

The results of the above-described studies were confirmed by Welch et al. (308) and Swihart and Welch (284), who found that hemolysins from *M. morganii*, *E. coli*, *P. vulgaris*, and *Pasteurella haemolytica* represented a closely related family designated MEPP hemolysins. By use of immunoblotting techniques, it was shown that *P. vulgaris* and *M. morganii* isolates produce a polypeptide similar in molecular size (110 kDa) and antigenically to *E. coli* HlyA hemolysin. These calcium-dependent pore-forming cytolysins are also known as RTX proteins, because of the presence of the 9-amino-acid repeat L-X-G-G-X-G-(N/D)-D-X in each of the toxins (RTX [repeat in toxins]) (309, 310).

The second family shares two strongly related *P. mirabilis* HpmA hemolysins (formerly described as cell-associated or cell-bound hemolysin) (123, 136, 142, 206, 252) and *Serratia marcescens* hemolysin (37, 309). The HpmA hemolysin, unrelated to *E. coli* HlyA cytolysin, was identified as a 166-kDa polypeptide common to *P. mirabilis* and *P. vulgaris* strains. By using immunoblots and DNA-DNA hybridization, it was shown that all 63 tested *P. mirabilis* strains and 23 of 24 tested *P. vulgaris* strains were able to produce calcium-independent hemolysin. These strains also hybridized with the *hpmA* gene probe and, in addition, the 166-kDa protein produced by them reacted with anti-HpmA antiserum. Moreover, the $HpmA^-$ mutant, which did not contain *hpmA* gene, had lost this hemolytic activity. The HpmA hemolysin was isolated from the supernatant of intensively aerated *P. mirabilis* cultures. This hemolysin appeared not to be stable, and it was detectable only if the hemolysin assay was immediately performed on fresh samples (284). Some *P. vulgaris* strains simultaneously expressed both calcium-dependent and calcium-independent hemolytic activities. Several investigated *Proteus* strains strongly hybridized to *Proteus hly* probes but did not produce calcium-dependent hemolytic ability, which shows that the *hly* operon is present but not expressed in these strains or that only small portions of the *hly* operon, not enough to express this type of hemolytic activity, are present.

The calcium-independent hemolysin determinant from a clinical isolate of *P. mirabilis* 477-12 was cloned, the DNA sequence of the 7,191-bp region was determined, and a functional characterization of products of the *hpm* genes was performed (161). It was found that two polypeptides, HpmA (166 kDa) and HpmB (63 kDa), are encoded by these loci and that the hemolysin genes are similar to the *S. marcescens* hemolysin genes *shlA* and *shlB*. Significant nucleotide identity (52.1%) was observed between the *hpm* and *shl* gene sequences. Strong similarities between the amino acid sequences of proteins A and B from these two bacteria were also observed (the respective sequence identities were 55.4 and 46.7%). The data mentioned above, as well as the presence of many conserved hydrophobic and amphipathic domains and predicted cysteine residues, indicate that *P. mirabilis* HpmA and *S. marcescens* ShlA hemolysins form a new hemolysin family among gram-negative opportunistic pathogens (289).

The studies of a biological function of these genes and corresponding proteins showed that HpmA did not require COOH-terminal domains for secretion. The HpmB proteins are responsible for transport and activation of hemolytic HpmA protein. HpmB is probably located in OM, whereas HpmA is located in the periplasm. In contrast to RTX proteins, HpmA is released with cleavage of the 29-amino-acid NH_2-terminal leader peptide sequence. HpmA hemolysin was produced at maximal level by *E. coli* DH1 harboring plasmid pWPM140 containing *hpmA* and *hpmB* genes, as well as by *P. mirabilis* strains, during the late logarithmic phase of growth (285). By construction of $HpmA^-$ mutants of two *P. mirabilis* strains, the distinct role of the amino-terminal part of the protein in the activity of HpmA was studied. It was shown that the in-frame deletion of the amino-terminal 140-kDa truncated HpmA led to the loss of its hemolytic activity. Moreover, it was also found that the first 355 amino acids of HpmA were required for secretion (285).

The role of HpmA hemolysin in UTI was studied by several authors. Peerbooms et al. (206, 208) showed that *P. mirabilis* strains synthesizing cell-associated hemolysin had a lower 50% lethal dose (LD_{50}) than nonhemolytic strains when injected intravenously into mice. It was also shown that hemolytic activity is correlated with cell invasiveness of *Proteus* strains (207, 238). Mobley and Chippendale (181) have found that strains isolated from patients with pyelonephritis or catheter-associated bacteriuria are not more active in the production of HpmA hemolysin than are fecal isolates, which suggested that production of this type of hemolysin is not a reliable index of virulence of *P. mirabilis*. HpmA was also cytotoxic against a variety of target cell lines: Daudi and Raji (human β-cell lymphoma), U937 (human monocytes), Vero, and cultured human renal proximal tubular epithelial cells (182, 285). In ascending murine UTI, in which *Proteus* strains were introduced into the bladder by urethral catheterization, mutant $HpmA^-$ behaved similarly to its parent strain in respect to kidney colonization and histopathological changes. The differences were found in the LD_{50} dose; mutant $HpmA^-$ had a sixfold higher LD_{50} than did the parent wild-type strains (285).

Quite a lot is known about the hemolytic activity of *P. penneri*. A collection of 45 strains of this genus were tested with respect to their cell-associated and cell-free hemolytic activities. The *P. penneri* strains synthesized extracellular and/or cell-bound hemolysins (239, 252). The cell-free hemolytic factor proved to be calcium dependent, and the kinetics of its production was similar to that of *E. coli* HlyA hemolysin. The maximal production of this hemolysin was observed in the logarithmic phase of growth, and thereafter its level was decreased (236, 252). The extracellular hemolysin of *P. penneri* strains was characterized as a polypeptide of approximately 110 kDa, characteristic of HlyA hemolysin. Senior (248) has found that *P. penneri* HlyA hemolysin was degradable by an EDTA-sensitive protease (probably the IgA protease produced by the same strain) to biologically inactive fragments. The results of genetic studies led to the suggestion that in *P. penneri* strains the *hly* determinant was localized on the chromosome, and, moreover, it showed partial homology between the *hly* determinants in *P. penneri* and *E. coli* (159). By using the colony hybridization technique, it was found that the alpha-hemolysin-like determinant was widely distributed among *P. penneri* strains (161). Only 1 of 71 strains of the *P. penneri* collection did not possess an *hlyA* locus (257). Cell-free *P. penneri* hemolysin reacted with polyclonal antiserum but not with monoclonal antibodies against *E. coli* HlyA hemolysin. These results indicate that extracellular hemolysin of *P. penneri*, although similar, differs in some epitopes from that present in *E. coli* α-cytolysin (161).

The presence of the *hpmA* genetic determinant and the production of the corresponding hemolysin were also examined in a collection of *P. penneri* strains (257). By use of *hpmA* and *hpmB* genetic probes, as well as HpmA-specific antiserum, it was shown that most *P. penneri* strains expressed HpmA

hemolytic activity. A 70% homology was found between *P. mirabilis* and *P. penneri hpm* determinants. Its localization in the *P. penneri* strains is similar (except for two strains) to that in *P. mirabilis* and *P. vulgaris* (257).

Synthesis of cell-free hemolysin by *P. penneri* strains is correlated with cytotoxic activity tested in vitro on Vero cell line and L-929 mouse fibroblasts (236, 239). The highest cytotoxic effect occurred in the most hemolytic $HlyA^+$ *P. penneri* 44; strain *P. penneri* 5 ($HpmA^+$ and $HlyA^+$) was less cytotoxic, whereas strain *P. penneri* 42 ($HpmA^+$) exhibited the weakest activity (236).

Proteus hemolysins belong to the family of pore-forming toxins (37). Recently, Benz et al. (28) have studied pore formation by *P. vulgaris* and *M. morganii* HlyA hemolysins and showed that both acted similarly to *E. coli* HlyA cytolysin. They formed transient ion-permeable, water-filled channels which are cation selective at neutral pH. The minimal diameter of these channels was estimated to be about 1 nm. It is most likely that HlyA hemolysins from all three species, *P. vulgaris*, *M. morganii*, and *E. coli*, oligomerize to form pores in lipid bilayer of membranes.

Siderophores

For a long time, it has been known that virtually all bacteria require soluble iron as an important nutritive compound. It is indispensable for growth and metabolism, mainly for most redox processes in all ecological systems: in the natural environment (soil, water), in artificial media, and in such niches as living organisms. In the presence of a deficiency of iron, bacteria produce iron chelators, named siderophores, which are excreted to the surroundings; they bind iron and transport it into the bacterial cells by using suitable receptor proteins and appropriate transport mechanisms. The synthesis of siderophores is under the control of chromosomal or plasmid genes.

In all kinds of the host-bacterium relationships (commensals and conventional and opportunistic pathogens), the bacteria are in competition with their host for iron. Eukaryotic proteins like transferrin and lactoferrin, with high iron affinity, render prokaryotic cells iron deficient. An efficient production of siderophores may seal the fate of an invader. From this point of view, they can be considered one of the virulence (invasiveness) factors.

In the family *Enterobacteriaceae*, most intensively studied for the production of siderophores, the situation is very differentiated. Certain species produce nearly exclusively the catecholate type (enterobactins) (201, 218), some produce the hydroxamate type (aerobactins) (80, 204), and others produce the recently described ferrioxamine type (30, 228) siderophores. In contrast to other members of the family *Enterobacteriaceae*, none of the *Proteus-Morganella-Providencia* (PPM) group produced the siderophores enumerated above. α-Hydroxyisovaleric acid has been described as a siderophore produced by *P. mirabilis* (73). Its general significance in amino-acid rich media is, however, disputable. The siderophore activity of α-keto acids, products of deamination of amino acids by these bacteria, was studied by Drechsel et al. (65). The production of different α-keto acids was monitored in a large collection of strains belonging to the PPM group. Their siderophore activity was studied by growth promotion and iron transport assays. The most significant siderophore activity was possessed by α-keto acids with aromatic or heteroaromatic side chains (phenylpyruvic acid from phenylalanine, or indolylpyruvic acid from tryptophan). Other α-keto acids with longer nonpolar side chains (α-ketoisocaproic or α-ketoisovaleric acid) also displayed the activity in question when smaller α-keto acids were lacking (like pyruvic, α-ketobutyric, or α-ketoglutaric acids). It must be stressed that α-keto acids do not form such stable complexes with ferric iron as hydroxamate siderophores do. Nevertheless, they are stable enough to function as iron chelators in the PPM group in the permanent absence of "classic," highly active siderophores. Deaminase-negative bacilli (*E. coli* and *Salmonella* spp.), used as control, are not able to use α-keto acids, which supports the view that a particular iron transport system has evolved in the PPM group. Especially, α-keto acids are easily obtained by L-amino acid deamination. The possibility of iron uptake in the course of one particular and unique metabolic reaction may support the colonization of host organisms by such opportunistic pathogens as *P. mirabilis*, *P. vulgaris*, and *P. penneri*, and in consequence it could be considered an important factor of the pathogenicity of these bacilli; however, it should be stressed that deaminases involved in this process are indispensable enzymes of bacterial fundamental metabolism.

Recently, Massad et al. (169) identified the *aad* gene encoding an amino acid deaminase (51 kDa; 473 amino acids) of a uropathogenic *P. mirabilis* strain. Its expression is not regulated by iron availability, since the nucleotide sequence upstream of *aad* does not contain the consensus Fur-binding site (iron box). Moreover, the activity of the amino acid deaminase was not affected by iron restriction in both *P. mirabilis*, as well as in *E. coli* carrying *aad* on a plasmid. It was also found that the amino acid deaminase activity was reduced by adding glucose to the bacterial growth medium, but this effect was not consistent with catabolite repression. Surprisingly, the *aad* gene probe did not hybridize with genomic DNA from *Providencia* or *Morganella* species, suggesting that these distinct genera produce deaminases encoded by the gene sequences unrelated to the *aad* gene of *P. mirabilis* or that these enzymes are conserved only at the level of the amino acid sequence.

CONCLUDING REMARKS

Bacteria belonging to the genus *Proteus* represent a particular group of microorganisms in the family *Enterobacteriaceae*. The name of the genus was given by Hauser (96) because the organisms reminded him of the Greek deity Proteus, who was able to change his shape, as these bacteria do. Now we know that the variable appearance of *Proteus* bacilli is related to the swarming phenomenon expressed by them and, moreover, that this ability seems to play a very important role not only in their colonization of solid surfaces but also in the pathogenicity of these bacteria. *Proteus* rods—the opportunistic pathogens—have been documented as virulent when certain conditions exist in humans and animals and are able to evoke infections in different regions of the body. Depending on the type of attacked tissue, *Proteus* bacilli mobilize virulence factors that are effective in adhesion to and penetration into the particular epithelial cells and in development of the infection syndrome. In general, *P. mirabilis* strains, which are less virulent than *E. coli*, are most often isolated from patients with recurrent UTI, from the urine of elderly, long-term catheterized patients (188, 304), and from men with anatomic abnormalities or with local damage of the urinary tract (281), and they are more common in young boys (29). However, it is well known that *P. mirabilis* will not cause bacteriuria unless an underlying defect is present in the host. Their virulence factors described above seem to act in a well-defined sequence (Fig. 6). It can be assumed that fimbriae and flagella are involved in the attachment of these bacilli to the epithelial tissue and colonization of the upper part of the urinary tract, respectively. The important role of

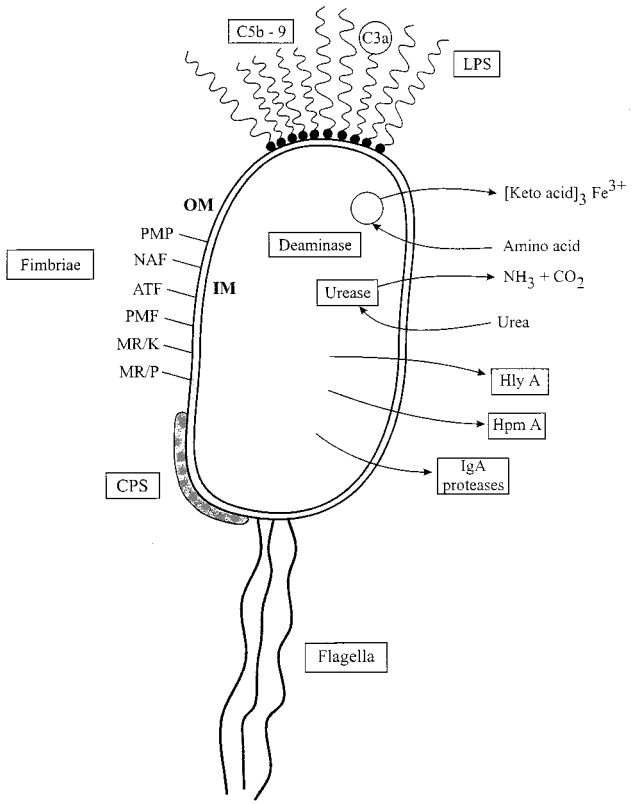


FIG. 6. Virulence factors of *Proteus* rods (180). Abbreviations: PMP, *P. mirabilis* P-like fimbriae; C5b-9 and C3a, complement components; IM, inner membrane. The other symbols are abbreviated in the text.

different types of fimbriae for pathogenesis of *Proteus* rods was documented in in vivo studies in mouse model. The most important types of fimbriae seem to be PMF and MR/P, which bind to the receptors on the bladder and renal epithelium, respectively. There is strong evidence from in vitro investigation that the swarming phenomenon and the antigenic variation of flagella may be significant in dissemination of bacteria during ascending UTI and in ensuring their survival in higher organisms. The most important pathogenic factor of *Proteus* bacilli seems to be urease—the enzyme hydrolyzing urea to NH_3 and CO_2, which results in elevation of pH in the surrounding of bacterial growth, favoring the production of IgA proteases. These enzymes destroy IgAs, the important immune system barrier in host tissue, and help bacteria to colonize there. The penetration ability of the host cells (invasiveness) is very important for the development of pathogenic processes caused by *Proteus* spp. The mechanism of this activity is not fully understood, although it was found that *P. mirabilis* can be internalized into the renal epithelium. It was suggested that internalization of bacteria as well as hemolysin- and urease-mediated cytotoxicity may play a role in the development of *Proteus* pyelonephritis. *Proteus* infections result in clinical complications including stone formation. According to the present knowledge, synthesis of urease and production of IgA proteases as well as capsule polysaccharides, most frequently structurally identical with the O-specific part of LPS, are very important for formation and localization of calculi in the tissue. The full repertoire of *Proteus* pathogenic factors is completed by different kinds of hemolysins (HpmA and HlyA) as well as endotoxin which act at the last stages of infection when it reaches the stages of sepsis and septic shock. Both hemolysins act directly cytotoxically against erythrocytes and the cells of the immune system. Moreover, HpmA and HlyA cytolysins, as well as endotoxin, cause inflammation due to the stimulation of host cells to produce different mediators, e.g., interleukins, TNF-α, and prostaglandins. There is much evidence that all *Proteus* virulence factors act in concert rather than individually. This could explain the fact that *Proteus* infections are very difficult to treat and still require additional studies, particularly in vivo.

The four most important open questions concerning *Proteus* virulence factors recently specified by Mobley and Belas (180) include (i) the specificity of the fimbrial adhesins and their real importance for virulence, (ii) the contribution of deaminases and IgA proteases in the pathogenicity, (iii) the role of antigenic variation of flagella in prevention of bacteria against the immune response, and (iv) the relevance of swarm cells in the colonization of the host tissue.

ACKNOWLEDGMENTS

We thank Harry T. Mobley and Robert Belas for providing Fig. 1 and 3, and we thank Janusz Włodarczyk for excellent technical assistance.

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